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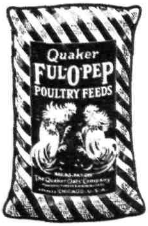

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Soybean Oil Meal Prepared at Different Temperatures as a Feed for Poultry[1]

J. W. HAYWARD, J. G. HALPIN, C. E. HOLMES, G. BOHSTEDT AND E. B. HART

Departments of Poultry Husbandry, Animal Husbandry, and Agricultural Chemistry, University of Wisconsin, Madison

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OSBORNE and Mendel (1917), Vestal and Shrewsbury (1932) report that cooking the soybean causes a very significant increase in its growth promoting properties for white rats. Robison (1930), and Vestal and Shrewsbury (1932, 1935) report similar results for swine. Hayward, Steenbock, and Bohstedt (1936) found in experiments with rats that heating the soybean to medium and high temperatures in the commercial methods of oil extraction practically doubles the nutritive value of the protein (grams of growth per gram of protein eaten). Raw beany tasting soybean oil meal prepared at a low temperature, gave about the same nutritive value for the protein as raw soybeans. In nitrogen balance studies these workers found that heating the soybean to high temperatures in the expeller method of oil extraction increased the digestibility of the protein about 3 percent and the biological value about 12 percent. Hayward, Bohstedt, and Fargo (1935) found pigs fed medium and high temperature soybean oil meal to make considerably greater daily gains with less feed required per unit of gain than for pigs receiving ground raw soybeans or raw tasting low temperature soybean oil meal. Robison (1930) reports similar results for different kinds of soybean oil meal. He assumes that the varied results obtained were due to the beans having received different heat treatments in the process of oil extraction.

The ineffectiveness experienced years ago with vegetable proteins in poultry rations was found to be largely due to mineral deficiencies (Wheeler, 1905, Kennard, Holder, and White, 1922, Kennard, 1924, and Philips and Hauge, 1925). For an excellent review of the literature pertaining to the mineral requirements of poultry, the reader is referred to a recent publication of Cruickshank (1935). Philips and Hauge (1925) found no difference for egg production between ground soybeans and soybean oil meal when each was properly supplemented with minerals. However, when the workers

[1] These studies were made possible by a fellowship supported by Allied Mills, Inc., Chicago, Ill., to whom we want to express our indebtedness and appreciation. Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

at Purdue University (1928) fed baby chicks raw soybeans plus minerals the results were not as satisfactory as when soybean oil meal and minerals were fed. Tomhave and Mumford (1933) also report unsatisfactory growth and a high feed requirement to produce a unit of gain, for chicks fed any large amount of ground raw soybeans. In recent nitrogen balance experiments with chicks, Wilgus, Norris, and Heuser (1935) determined the relative protein efficiency of various protein supplements. They found the proteins of ground raw soybeans and soybean oil meal to be 58 and 88 percent, respectively, as efficient as casein. They have assumed that the increase in protein efficiency for the soybean oil meal was caused by heating the soybean in the process of oil extraction.

It is apparent from the review of the literature that little if any attempt has been made to trace exact history of heat treatment of the soybean and determine the effect of heating the soybean in the commercial method of oil extraction upon the value of soybean oil meal in practical poultry rations. The figures[2] for bushels of soybeans harvested as seed in the United States and the production of soybean oil meal during the past few years reveal the increased popularity of the soybean and its products in this country. These figures also suggest that a supply of soybean oil meal will be available in ever increasing amounts in the future. Apparently, this meal is destined to be used extensively in rations for poultry. Therefore, information on the effect that the oil extraction temperature has upon the feeding value of soybean oil meal for poultry is important.

EXPERIMENTAL

Production of Experimental Material: Commercially, soybean oil is extracted by the expeller, the hydraulic, or the solvent processes. The resulting meals are known according to the method of extraction employed. Each kind of soybean oil meal, produced where possible at low, medium, and high temperatures were included in the experimental materials. The production of the desired samples was arranged with the respective soybean processing companies in order to obtain a complete history of the various heat treatments used in their preparation. The data pertaining to the temperatures used in the production of the commercial meals are given in Table 1. In addition to the various samples of soybean oil meals, a supply of the soybeans was reserved. These soybeans were for the most part of the Illini variety.

Feeding Experiments: For convenience the experiments reported herein have been grouped into two separate series, namely, series 1 and 2. In all feeding trials considered under series 1 and 2, the same basal

[2] These figures for bushels of soybeans harvested as seed were obtained from the U. S. Department of Agriculture, Div. of Crops and Livestock Estimates. The figures for the yearly production of soybean oil meal were supplied by the U. S. Department of Agriculture, Bureau of Agricultural Economics.

Bushels Soybeans Gathered

Crop Year Oct. 1 to Sept. 30	Total bushels for United States
1929-30	8,670,000
1930-31	12,217,000
1931-32	15,463,000
1932-33	13,121,000
1933-34	11,177,000
1934-35	18,627,000
1935-36	39,637,000

Tons Soybean Oil Meal Produced

Crop Year Oct. 1 to Sept. 30	Total tons for United States
1929-30	39,994
1930-31	97,659
1931-32	113,399
1932-33	82,383
1933-34	72,879
1934-35	221,871
1935-36	599,855

TABLE 1.—*History of preparation of soybean oil meals**

Expeller meals

Degree of heat treatment	Preliminary drying		Conveying		Prewarming		Expelling the oil	
	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
	°C.	Min.	°C.	Min.	°C.	Min.	°C.	Min.
low	100–112	8	82–90	7	90	13	cold shaft 105	2.0
medium	100–112	8	82–90	7	100–112	13	hot shaft {112–125	1.5
							{130	1.0
high	100–112	8	82–90	7	100–112	13	hot shaft {140	1.5
							{150	1.0

Hydraulic meals

Degree of heat treatment	Preliminary drying		Heating		Cooking		Pressing	
	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
	°C.	Min.	°C.	Min.	°C.	Min.	°C.	Min.
low	60–80	60	60	10	82	90	75–65	50–60
medium	60–80	60	60	10	105	90	75–65	50–60
high	60–80	60	60	10	121	90	80–68	50–60

Solvent meal

Preliminary heating		Extracting	Drying and cooking		Aerating and cooling	
Temperature	Time	Temperature	Temperature	Time	Temperature	Time
°C.	Min.	°C.	°C.	Min.	°C.	Min.
60	10	45	98	15	78–45	10–20

* The expeller meals were prepared in the Soybean Plant of Allied Mills, Inc., at Peoria, Ill., with Anderson R. B. Expellers. The hydraulic and solvent meals were prepared in the Soybean Plants of the Archer-Daniels-Midland Co. at Toledo, Ohio, and Chicago, Ill., respectively.

ration has been fed. It consisted of the following ingredients:

Basal ration

Ground yellow corn	45 parts
Wheat bran	15 parts
Standard middlings	15 parts
Alfalfa hay meal	5 parts
Limestone grit	3 parts
Salt (iodized)	0.5 part
Cod liver oil	1 part
Total	84.5 parts by weight

In all of the feeding trials of series 1 and 2, where minerals were added to approximate the calcium and phosphorus contained in the control ration fed to lot 1, the following mixture was used:

Mineral mixture

Steamed bone meal	3 parts
High calcium limestone (finely ground)	1 part

In series 1 feeding trials were conducted to determine the effectiveness for growth of chicks and pullets, and for production and hatchability of eggs, of different levels of soybean oil meal with the combination of ingredients which were used. It was thought necessary to have this sort of information, especially that pertaining to growth of baby chicks, before conducting experiments with soybean oil meals produced at different temperatures.

For the growth feeding trial, experiment 1, series 1, started April 25, 1934, 357

TABLE 2.—*The effect of different levels of soybean oil meal upon growth, livability, and feed requirements of chicks. Experiment 1, Series 1*

Lot	Ration		Chicks started	Average weight in grams								Average lb. feed consumed per lb. of gain	Deaths
				Initial	2 weeks	4 weeks	6 weeks	8† weeks	12 weeks	16 weeks	20 weeks		
	Basal	84											
	Meat scraps	8											
1	Dried milk	8	51	39	101	204	348	499	870	1245	1595	5.0	1
	Basal	83											
	Meat scraps	4											
	Dried milk	4											
	Soybean oil meal*	8											
2	Mineral mixture	1	51	40	92	189	331	479	896	1235	1600	5.4	2
	Basal	82.5											
	Meat scraps	2											
	Dried milk	2											
	Soybean oil meal*	12											
3	Mineral mixture	1.5	51	39	89	191	328	459	801	1200	1553	5.0	0
	Basal	81											
	Dried milk	3											
	Soybean oil meal*	14											
4	Mineral mixture	2	51	40	90	187	316	463	855	1193	1553	5.7	0
	Basal	82											
	Soybean oil meal*	16											
5	Mineral mixture	2	51	40	77	146	245	365	706	1062	1377	5.9	0
	Basal	91											
	Meat scraps	4											
	Dried milk	4											
6	Mineral mixture	1	51	40	85	174	304	413	769	1119	1408	5.4	0
	Basal	95											
	Meat scraps	2											
	Dried milk	2											
7	Mineral mixture	1	51	39	78	152	270	381	676	995	1317	5.7	1

* High temperature expeller soybean oil meal, processed at 140-150°C. for 2½ minutes.
† Pullets only.

Single Comb White Leghorn day-old chicks were taken from the pedigree baskets and distributed into seven lots. Chicks were brooded in small brooder coops equipped with electric hovers. Each lot had access to a sand and gravel yard built over a cinder base. Chicks were weighed weekly during the first few weeks and on even weeks thereafter. Cockerels were removed at eight weeks and the pullets continued on the ration.

The data for the growth trials of experiment 1 are contained in Table 2. Lot 1 was fed Wisconsin Station Ration No. 2, which is considered complete for baby chicks in every respect. This ration consists of the basal ration plus 8 parts of meat scraps and 8 parts of dried milk. In lots 2, 3, 4, and 5 high temperature expeller soybean oil meal and the mineral mixture were added in sufficient amounts to approximate the protein and minerals (Ca. and P.) contained in the ration fed to lot 1. This ration (control) contained about 17 percent of protein, 1.93 percent calcium, and .92 percent phosphorus.

The soybean oil meal which was fed to lots 2, 3, 4, and 5 was high temperature expeller meal, the same that gave such satisfactory results in previous experiments with rats (Hayward, Steenbock, and Bohstedt, 1936), and swine (Hayward, Bohstedt, and Fargo, 1935). Lots 6 and 7 received the same amount of the animal proteins as fed lots 2 and 3, respectively. Naturally in these lots, lots 6 and 7, protein levels were disregarded. However, sufficient amounts of the mineral mixture were added to approximate the calcium and phosphorus content of the check lot ration. It is apparent that lots 6 and 7 are very important in that they serve as a negative control to lots 2 and 3. In this regard it is believed that many feeding trials have been reported intending to demonstrate the value of certain protein feeds when as a matter of fact the investigators have used such a high level of animal protein in the basal ration that good results could be expected without further supplement.

As seen from the results in Table 2, the expeller soybean oil meal, processed at a high temperature, when supplemented with minerals so as to maintain the level of calcium and phosphorus in the ration at 1.93 percent and .92 percent, respectively, gave satisfactory growth with a less satisfactory feed requirement for each unit of gain (except for lot 3) when it was substituted for one-half, three-fourths, or less of the animal protein (lots 2, 3, and 4). The same, however, cannot be said for lot 5 where soybean oil meal plus minerals replaced all of the animal protein. Here the rate of growth was considerably less and feed requirement greater than for the lots receiving 8 parts of meat scraps and 8 parts of dried milk or a combination of meat scraps, dried milk, and soybean oil meal (lots 1, 2, 3, and 4). The viability of the chicks and pullets was satisfactory for all lots except lot 5. A check on the early chick losses in lot 5 was made during the summer of 1934. Two lots of 55 chicks each were fed for six weeks in this trial. One lot received the same ration as lot 1 and the other lot the same ration as lot 5. The mortality was comparable, there being two deaths in each of the two lots. This suggests that the greater loss of chicks in lot 5, experiment 1, series 1, was perhaps due to other causes than the ration.

The pullets in experiment 1 were sorted the middle of September for uniformity, reduced to an even dozen for each lot, and moved to comparable laying quarters where trap nest records were kept of their egg production for 11 months. The rations fed each lot were the same as during the growth period except that oyster shells were fed ad libitum. The pullets had access to clean drinking water at all times.

TABLE 3.—*The effect of different levels of soybean oil meal upon egg production. Experiment I, Series I. Twelve pullets to each lot.*

Lot	Ration		Av. age in days to first egg	Average egg production per hen											Total 11 months
				Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	
1	Basal Meat scraps Dried milk	84 8 8	174	15.4	17.8	11.6	13.0	14.0	19.9	20.9	22.3	19.0	21.9	16.6	192.4
2	Basal Meat scraps Dried milk Soybean oil meal* Mineral mixture	83 4 4 8 1	177	11.0	21.3	15.5	15.1	13.0	18.2	22.2	22.8	19.7	19.1	16.6	194.5
3	Basal Meat scraps Dried milk Soybean oil meal* Mineral mixture	82.5 2 2 12 1.5	182	11.4	19.7	18.6	17.3	14.0	19.9	18.9	17.7	15.0	15.9	15.4	183.8
4	Basal Dried milk Soybean oil meal* Mineral mixture	81 3 14 2	179	11.0	20.7	15.1	14.6	11.9	16.0	20.8	19.1	18.0	17.2	15.0	179.4
5	Basal Soybean oil meal* Mineral mixture	82 16 2	182	7.7	17.3	14.1	7.7	9.9	16.5	17.8	17.9	16.0	15.5	15.0	155.4
6	Basal Meat scraps Dried milk Mineral mixture	91 4 4 1	191	5.0	12.9	13.8	15.7	15.2	10.1	15.3	15.3	11.6	10.1	11.0	136.0
7	Basal Meat scraps Dried milk Mineral mixture	95 2 2 1	191	3.0	16.0	13.2	13.0	15.0	16.8	13.2	15.9	11.1	10.3	7.9	135.4

* High temperature expeller soybean oil meal, processed at 140-150°C. for 2½ minutes.

The data for this egg production trial experiment 1, series 1, which are given in Table 3, show a similar average egg production for the pullets fed 8 parts meat scraps and 8 parts dried milk (lot 1) and the three combinations of soybean oil meal with animal protein and mineral additions (lots 2, 3, and 4). However, when soybean oil meal plus minerals replaced all of the animal protein (lot 5) or when the low protein negative control rations were fed (lots 6 and 7), the average egg production was decidedly inferior to the production for the higher protein groups. The average age in days for the first egg laid is nearly normal for all groups. Other records show that 170 to 180 days for the first egg was about normal for this strain of Single Comb White Leghorns. However, 191 days as in lots 6 and 7 would not be considered abnormal.

The feeding trials considered in experiment 2, series 1, were started April 24, 1935. Three hundred eighty-five Single Comb White Leghorn day-old chicks were taken from the pedigree baskets and distributed into seven lots. The chicks were raised in the same equipment as the 1934 lots in experiment 1. The data for these feeding trials (experiment 2) are contained in Table 4. With the exception of lots 6 and 7, this trial is identical with the growth trials of experiment 1. Lot 6 in experiment 2 received the same level of soybean oil meal as lot 5 but the ration differs in that 8 parts of meat scraps and 8 parts of dried milk were added. These protein supplement additions brought the protein content of the ration up to about 22 percent whereas the protein in the rations fed all other lots was about 17 percent. Mineral additions were not made since this ration fed to lot 6 supplied more calcium and phosphorus than the control ration fed to lot 1. The ration fed to lot 7 contained a sufficient amount of the solvent extracted soybean oil meal, cooking temperature 98°C., and the mineral mixture to equal the protein and minerals (Ca. and P.) contained in the control ration fed to Lot 1 and the rations fed to all other lots except lot 6. This solvent extracted soybean oil meal had been found to contain proteins with a high nutritive value in experiments of Hayward, Steenbock, and Bohstedt (1936). Since this solvent meal had received the lowest processing temperature of all meals having a high nutritive value for rats it was thought desirable to include it in these poultry experiments especially in view of the contention of some investigators (Byerly, Titus, and Ellis, 1933) that the high temperatures used in processing soybeans had destroyed certain nutritive properties in the resulting soybean oil meal.

As seen in Table 4 these feeding trials, experiment 2, series 1, resulted in about the same comparative growth for lots 1, 2, 3, and 4 as the same lots in experiment 1. The feed required per unit of gain for lots 2, 3, and 4 in experiment 2 compared favorably with the feed requirement for lot 1. The chicks in lot 5 receiving the high temperature expeller soybean oil meal and the mineral mixture replacing all of the animal protein of the control ration fed to lot 1, while showing relatively poor growth and economy of gains, made greater growth with less feed required per unit of gain than for a similar ration fed lot 5 in experiment 1. Lot 6 with a high protein intake made greater growth on less feed required per unit of gain than any of the lots in this feeding trial. The chicks receiving the solvent extracted soybean oil meal (lot 7) replacing the high temperature expeller soybean oil meal in a ration similar in all other respects to the ration fed to lot 5 surpassed the all soybean oil meal group (lot 5) and equalled, in average weight of pullets at 20 weeks but required more feed per unit of gain than the all animal protein control lot (lot 1). The significance of these results for

TABLE 4.—*The effect of different levels of soybean oil meal upon growth, livability, and feed requirement of chicks. Experiment 2, Series 1.*

Lot	Ration		Chicks started	Average weight in grams								Average lb. feed consumed per lb. of gain	Deaths
				Initial	2 weeks	4 weeks	6(†) weeks	8 weeks	12 weeks	16 weeks	20 weeks		
1	Basal Meat scraps Dried milk	84 8 8	55	39	107	246	406	616	1056	1275	1668	4.8	0
2	Basal Meat scraps Dried milk Soybean oil meal* Mineral mixture	83 4 4 8 1	55	39	95	239	375	533	929	1199	1660	5.1	0
3	Basal Meat scraps Dried milk Soybean oil meal* Mineral mixture	82.5 2 2 12 1.5	55	39	90	234	339	518	888	1189	1578	4.9	1
4	Basal Dried milk Soybean oil meal* Mineral mixture	81 3 14 2	55	39	93	223	382	581	955	1261	1647	4.9	0
5	Basal Soybean oil meal* Mineral mixture	82 16 2	55	39	82	178	314	469	860	1184	1581	5.5	1
6	Basal Meat scraps Dried milk Soybean oil meal*	68 8 8 16	55	39	113	286	468	698	1062	1322	1771	4.6	0
7	Basal Soybean oil meal** Mineral mixture	83 15 2	55	39	86	202	346	491	838	1151	1699	5.0	0

* High temperature expeller soybean oil meal, processed at 140-150°C. for 2½ minutes.
** Solvent extracted soybean oil meal, cooked, at 98° for 15 minutes.
† Pullets only.

the solvent soybean oil meal ration fed to lot 7, however, must be determined in future experiments. Although the data for hatchability of eggs are available for the pullets saved for egg production trials from experiment 1, it will not be published until the same for pullets from experiment 2 and perhaps future experiments are completed.

TABLE 5.—*The effect of the heat used in processing the soybean upon the efficiency of the soybean oil meal protein for growing chicks. Experiment 1, Series 2*

Lot	Ration	Chicks started	Initial	Average weight per chick (grams)				Average lb. feed consumed per lb. gain	Deaths
				2 weeks	4 weeks	6 weeks	8 weeks		
1	Basal 84 Meat scraps 8 Dried milk 8	56	39	103	236	429	684	2.9	0
2	Basal 82 Soybean oil meal 16 Expeller—temperature 105°C. (2 min.) Mineral mixture 2	56	40	68	123	199	281	4.2	1
3	Basal 82 Soybean oil meal 16 Expeller—temperature 140°C.-150°C. (2½ min.) Mineral mixture 2	55	40	90	183	299	468	3.6	3
4	Basal 82 Soybean oil meal 16 Hydraulic—temperature 82°C. (90 min.) Mineral mixture 2	55	39	75	151	255	392	3.7	4
5	Basal 82 Soybean oil meal 16 Hydraulic—temperature 121°C. (90 min.) Mineral mixture 2	56	39	90	188	317	445	3.5	1

Series 2 consists of feeding trials (experiments 1 and 2) which were conducted to determine the effect of the heat used in processing the soybean upon the efficiency of the protein contained in soybean oil meal for growing chicks. Experiment 1 contained five lots of Single Comb White Leghorn chicks which were started on the experimental rations April 10, 1935. All lots were brooded under comparable conditions. The rations fed each lot and other data for this experiment are contained in Table 5. The soybean oil meal rations fed lots 2, 3, 4, and 5 were formulated to equal as near as possible the control ration fed lot 1, in total protein and minerals (Ca. and P.). This control ration contained about 17 percent of protein, 1.93 percent of calcium, and .92 percent of phosphorus.

As seen from the results in Table 5, the feeding of the expeller soybean oil meal processed at a high temperature (140-150°C. for 2½ minutes) resulted in chicks weighing about twice as much at 8 weeks, with less feed required per unit of gain, as the chicks fed the expeller soybean oil meal processed at a low temperature (105°C. for 2 minutes). An advantage was also found in total weight of chicks at eight weeks and feed requirement for the hydraulic soybean

oil meal cooked at a high temperature (121°C. for 90 minutes) over the hydraulic soybean oil meal cooked at a low temperature (82°C. for 90 minutes). This advantage, however, for the high over the low temperature was not as significant for the hydraulic meals as it was for the meals prepared by the expeller method of oil extraction. The results show that the proteins contained in the high temperature hydraulic soybean oil meal and the high temperature expeller soybean oil meal were equally efficient in growing baby chicks. However, lot 1, fed the control ration containing only animal protein supplements shows a still more rapid rate of growth with less feed required to produce a unit of gain than any of the soybean oil meal lots. All of the comparative results for the expeller and hydraulic soybean oil meals prepared at low and high temperatures agree very closely with the results that Hayward, Steenbock, and Bohstedt (1936) obtained in experiments with white rats. They found these high temperature expeller and hydraulic soybean oil meals to contain proteins with a nutritive value about twice that for the proteins of these low temperature expeller and hydraulic meals.

Experiment 2, series 2, was started May 15, 1935. The chicks used were day-old Single Comb White Leghorns. All lots were

TABLE 6.—*The effect of the heat used in processing the soybean upon the efficiency of the soybean oil meal protein for growing chicks. Experiment 2, Series 2*

Lot	Ration		Chicks started	Average weight per chick in grams					Average lb. feed consumed per lb. gain	Deaths
				Initial	2 weeks	4 weeks	6 weeks	8 weeks		
1	Basal Meat scraps Dried milk	84 8 8	31	40	105	243	440	653	3.1	1
2	Basal Meat scraps Dried milk Ground raw soybeans Mineral mixture	80.5 2 2 14 1.5	31	41	73	150	247	278	5.2	4
3	Basal Meat scraps Dried milk Soybean oil meal Expeller—temperature 140°-150°C. (2½ min.) Mineral mixture	82.5 2 2 12 1.5	30	40	99	212	392	560	3.5	1
4	Basal Meat scraps Dried milk Soybean oil meal Hydraulic—temperature 105°C. (90 min.) Mineral mixture	82.5 2 2 12 1.5	30	40	94	231	382	516	3.7	0
5	Basal Meat scraps Dried milk Soybean oil meal Solvent—temperature 98° C. (15 min.)	82.5 2 2 12 1.5	31	40	96	203	357	510	3.5	0

brooded under comparable conditions. The rations and other data for this experiment are contained in Table 6. The soybean and soybean oil meal rations fed to lots 2, 3, 4, and 5 were formulated, as in the preceding experiment of this series, to approximate the control ration (lot 1) in content of protein, calcium, and phosphorus. In these feeding trials the high temperature expeller soybean oil meal produced somewhat more rapid gains than either the hydraulic meal cooked at medium temperature (105°C. for 90 minutes), or the solvent extracted meal. The feed required per unit of gain for the high temperature expeller and the solvent extracted soybean oil meal was the same and slightly less than the feed requirement for the lot fed the medium temperature hydraulic soybean oil meal. The ground raw soybeans, however, grew the chicks much slower, with a significantly greater feed requirement per unit of gain than the soybean oil meals. These results suggest that the heating of the bean in preparing these meals has definitely increased the efficiency of their proteins for growing baby chicks.

The importance of properly heating the soybean to render the protein more efficient for growing chicks is most significantly brought out in these feeding trials (experiment 2, Table 6) since the soybean and soybean oil meal supplements were fed in combination with animal protein. Although the growth obtained at eight weeks with these properly heated soybean oil meals when fed in combination with 2 parts of meat scraps and 2 parts of dried milk plus minerals were not equal to the results obtained when 8 parts of meat scraps and 8 parts of dried milk formed the protein supplement, the results for the pullets at 20 weeks of age (experiments 1 and 2, series 1, Tables 2 and 4) and the results for egg production (experiment 1, series 1, Table 3) where similar meals were fed do suggest that this combination of protein supplements plus minerals is adequate for poultry rations.

SUMMARY

High temperature expeller soybean oil meal (processed at 140-150°c. for 2½ minutes), medium and high temperature hydraulic soybean oil meal (cooked at 105° and 121°C. for 90 minutes), and solvent extracted soybean oil meal (cooked at 98°C. for 15 minutes) resulted in chicks weighing about twice as much, with less feed required per unit of gain, as the chicks fed ground raw soybeans and low temperature expeller soybean oil meal (processed at 105°C. for 2 minutes) in feeding trials conducted for eight weeks.

The medium and high temperature hydraulic soybean oil meals were definitely superior, in growth produced and feed required, to the low temperature hydraulic soybean oil meal (cooked at 82°C. for 90 minutes). However, this low temperature hydraulic meal was not so decidedly lacking in growth promoting properties as were the ground raw soybeans and low temperature expeller soybean oil meal.

The chicks fed a ration containing 16 parts soybean oil meal and minerals, or a combination consisting of 12 parts soybean oil meal, 2 parts meat scraps, and 2 parts dried milk plus minerals, did not equal in average weight at eight weeks or in feed required per unit of gain the chicks fed a protein supplement consisting of 8 parts meat scraps and 8 parts of dried milk, regardless of the soybean oil meal that was used.

As for the chicks fed the soybean oil meals which have been designated as properly heated, 12 parts soybean oil meal, 2 parts meat scraps, and 2 parts dried milk plus minerals was superior in results obtained in these eight-week feeding trials to 16 parts soybean oil meal plus mineral.

The combination of 12 parts high temperature expeller soybean oil meal, 2 parts

meat scraps, and 2 parts dried milk plus minerals surpassed 16 parts of soybean oil meal plus minerals and approximately equalled the combination of 8 parts soybean oil meal, 4 parts meat scraps, and 4 parts dried milk plus minerals or the all animal protein supplement consisting of 8 parts meat scraps and 8 parts dried milk in weight of pullets and feed required per unit of gain at 20 weeks in two feeding trials. This combination of 12 parts high temperature expeller soybean oil meal, 2 parts meat scraps, and 2 parts dried milk plus minerals also proved highly efficient in comparison to other supplements tested in producing eggs in one 11-month trial reported for pullets.

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Titre Fluctuation in Agglutination Tests for Pullorum Disease in Hens

B. H. Edgington
Ohio Agricultural Experiment Station, Department of Animal Industry, Animal Disease Laboratories, Reynoldsburg, Ohio

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Different investigations have shown that the agglutinin titre of the blood serum of some *S. pullorum* infected adult fowls shows fluctuations in tests of the same birds conducted over a period of several months.

Beach, J. R. (1927) ran monthly agglutination tests over a period of one year beginning with 70 reactor pullets. The change from positive to negative reactions observed in these birds at different tests was not thought to be a progressive decrease of agglutinins the result of birds ceasing to react, since at later tests there was an increase in the total number of reactors. Beach, B. A. (1927) tested a group of hens 11 times during a period of 13 months. Titre fluctuations occurred in several of the birds. Newson, Cross and Ufford (1928) in 21 tests conducted during a period of approximately three years, had 21 birds in a group of 59 give fluctuating tests. They were of the opinion that these inconsistencies "appeared to be resident in the agglutinin content of the blood." Kernkamp (1929) reported agglutinin titre fluctuations in a group of birds subjected to repeated tests. Edwards and Hull (1929) during a year ran tests at monthly intervals on a group of 93 reactor hens and observed but slight variations in the tests. Only six out of a total of 984 tests were negative.

What influence birds showing such fluctuations of agglutinin titre might have in flocks wherein an effort is being made to eliminate pullorum disease is uncertain.

Birds showing titre fluctuations varying in value from that of reactor to non-reactor and again back to reactor, are of particular interest. It is evident that tests might be conducted at a time when the birds were showing non-reactor titres and thus permit their retention in otherwise supposedly non-infected flocks. Such variations probably are not common. However, their occurrence stimulates interest in seeking the cause of such fluctuations.

It seems reasonable to assume that the elimination of infection from reactor birds would result in a recession of their agglutinin titres and if the fowls be subjected to reinfection their titres would increase.

An opportunity for *S. pullorum* transmission by association of non-infected with infected adult birds, appears to exist. A number of investigators have shown that adult fowls may acquire the infection through association with reactor birds.

Edwards and Hull (1929) allowed 73 reactor and 15 non-reactor hens to associate for one year in a pen having an adjacent lot. Five of the 11 birds that lived throughout the period became infected with *S. pullorum*. Warrack and Dalling (1931) held six non-reactor birds and eight reactors in direct pen association for 18 months without transmission of infection. However, in a second test with six reactor and two non-reactor birds kept in more restricted quarters, both negative birds became reactors. Bunyea (1935) allowed 12 non-reactor pullets to associate with 35 reactor pullets for

TABLE 1 —*Agglutinin titres of pen group*

Bird No.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
1.	0	0	0	0	25p	0	0	0	0	0	0	0
2.	0	0	0	0	200	200	200	200	200p	200p	200p	50
3.	0	25	0	0	0	0	0	0	0	0	0	0
4.	0	25	0	0	0	25p	25p	25p	25p	25p	0	25p
5.	0	0	0	0	0	0	0	0	0	0	0	0
6.	0	0	0	0	25	0	0	0	0	0	0	0
7.	0	0	0	0	0	0	0	0	0	0	0	
8.	0	0	0	0	200	200	100p	400p	200p			
9.	0	0	0	0	0	0	0	0				
10.	0	0	0	0	0	0	0					
11.	0	0	0	0	0							
12.	0	0	0	0	200							
13.	0	0	0	0								
14.	0	25	0									
15.	25	25	0	100p	0	0	0	25p	25p	25p	0	0
16.	25	50	25p	25	25	25	25p	25p	25p	25p	25	25p
17.	25	50	50	50	25	100p	100p	50				
18.	25	25	25									
19.	50	25	25p	25p	25	25p	25p	50p	50p	50p	50p	50p
20.	50	100	50	50	100	200p	200p	200p	200p	200p	100p	100p
21.	50	25	50	25	50	50	50p	50p	50p	50p	100p	25p
22.	50	50	25	25								
23.	50	50	100									
24.	100	100	50	100	100	400	400	400	400	200	200	200
25.	100	100	100	200	800	200	800	800	800	400	200	400
26.	100	100	50	100	50	200	200	200	200	200	100	100
27.	100	100	100	25	200	200	100	400	400	400	200	100
28.	100	100	100	100	100	200	200	400	400	200	100	100
29.	100	0	0	100	400	800	800	1600	800	800	400	800
30.	100	100	25	25p	200	100	200	200	100	50p	100	25p
31.	100	100	200	100	100	200	400	200	100	200		
32.	100	100	200	100	200	400	400	800	400	400		
33.	100	100	25	25	50	100	50	50	100			
34.	100	100	50	50	100	100	200	100				
35.	100	100	50	50	100	200	200					
36.	100	25	25	25	200							
37.	100	50	50	100								
38.	200	800	400	800	1600	1600	3200	3200	1600	6400	1600	800
39.	200	200	200	200	400	1600	1600	800	800	400	400	400
40.	200	400	200	400	800	1600	1600	1600	800	800	800	400
41.	200	800	800	1600	1600	3200	800	800	1600	1600	800	1600
42.	200	400	100	200	400	800	200	800	400	400	200	
43.	200	200	400	400	200	400	200	50				
44.	200	200	100	100	50	50						
45.	200	100	50	50	200	100						
46.	200	100	50	25	50							
47.	200	200	200									
48.	1600	1600	800	400	400	1600	800	800	400	800	400	400
49.	800	200	200	400	400	1600	800	3200	3200	800	800	

NOTE: Numbers alone—represent highest dilution in which agglutination was complete.
Numbers followed by p—indicate but partial agglutination in that dilution.

seven months. The groups were then separated and the pullets mated to non-reactor males. Six of the non-reactor pullets were proved to harbor pullorum infection.

The object of the present experiment was to obtain information regarding the influence, if any, pen association of adult fowls might have upon titre fluctuations as observed in agglutination tests conducted at monthly intervals.

The birds used in the experiment were cull or reactor hens obtained from four different flocks, two of which were composed of Single Comb White Leghorns, one of

TABLE 2.—*Agglutinin titres of cage group*

Bird No.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.
1.	0	0	0	0	0	0	0	0	0	0	0	0
2.	0	0	0	0	0	0	0	0	0	0	0	0
3.	0	0	0	0	0	0	0	0	0	0	0	0
4.	0	25	25p	800	1600	800	400	800	3200	1600	1600	200
5.	0	25	25	0	0	0	0	0	0	0	0	0
6.	0	0	0	0	0	0	0	0				
7.	0	25	25	50	25	25						
8.	0	0	0	0	0							
9.	0	0	0	0	0							
10.	0	0	0	0								
11.	0	0	0									
12.	0	0	0									
13.	0	0	0									
14.	25	25	25	25p	0	25p	0	25p	50p	50p	25p	25p
15.	25	50	50	100	100	200						
16.	25	50	25	0								
17.	25	200	200									
18.	50	100	50	50	800	800	800	200	400	100	50p	50
19.	50	25	0	25	50	25p	25p	25p	25p	25p	25p	25p
20.	50	100	50	25	0	200	200	200	200	400	200	100
21.	50	50	50	50	25	25p	25p	25p	25p	50p	50p	25p
22.	50	50	25	50	25	50	50p	25p	25	50p	25	
23.	50	25	50	25	1600	400						
24.	50	25	50	50	25	25						
25.	100	50	50	50	100	100	50	100	200	100	100	100
26.	100	200	50	50	50	50	50	100	100	50	50	50
27.	100	200	200	200	200	200	100	100	100	200	100	100
28.	100	100	25	25	50	50	50p	50p	50	50p	25p	25p
29.	100	200	100	200	100	100	200	800	400	200	400	200
30.	100	50	25	25	25	200	400	200	200	200	50	50
31.	100	100	50	25	50	200	400	400	400	800	100	100
32.	100	100	100	50	100	100	50	50	50	100	100	100
33.	100	200	100	200	200	200	1600	1600	400	1600	400	400
34.	100	50	25	50	100	200	25	400	50	200		
35.	100	100	25	50	25	50p	25p	25p	0			
36.	100	50	25	25	200	200	400	400	400			
37.	100	100	50	50	50	100	100	50				
38.	100	100	50	25	50	50	50p					
39.	100	100.	50	50								
40.	100	200	100	100								
41.	200	100	100	200	200	100	200	400	100	200	100	100
42.	200	100	100	50	200	200	200	200	100	200	100	200
43.	200	200	100	100	100	800	200	100	1600	1600	800	400
44.	200	200	100	50	50	100	100	100				
45.	200	100	100	50	50	100						
46.	200	100	50									
47.	400	1600	1600	3200	3200	6400	3200	3200	3200	1600	3200	3200
48.	400	400	200	200	800	800	800	800	400	1600	200	400

Barred Rocks, and one of Rhode Island Reds. The birds were combined into two groups, the uniformity of which was based on the origin and the agglutinin titres of the fowls as determined by their first test.

The "pen" group consisted of 49 hens which were held in a single pen that permitted freedom of contact by the various members of the group.

The "cage" group was composed of 48 hens. Each bird of this group was confined to an individual cage having a wire grille floor which readily permitted droppings to pass beyond reach of the hen and so inclined that eggs laid would roll into an outside receptacle. Each cage had an individual feed container but the watering pans were common for two adjacent cages.

Agglutination tests were made at monthly intervals for a period of one year. The strains of *S. pullorum* used in the preparation of the antigen were subcultures of three strains originally obtained from the Antigen Committee of the Conference of Laboratory Workers in Pullorum Disease Control. The antigen was standardized to the No. 1 tube of the McFarland scale. Tables 1 and 2 show the agglutinin titre limits of the birds in the two groups.

Since the test birds were selected from *S. pullorum* infected flocks, titre increases may have resulted in either group from infections that occurred prior to the beginning of this experiment. However, recessions in titre could not be attributed to such factors.

If exposure incident to the association of pullorum reactor hens in the same enclosure was to influence the agglutinin titres of the birds, the anticipated trend of tire change would be an increase, whereas the reverse should be expected in birds protected from such exposure by isolation in individual cages.

In each group were birds showing negative and others giving positive reactions throughout the entire test period. Others developed and maintained high titres. But in no instance did any bird showing agglutinins at the first test, in any dilution used, become consistently negative.

Titre variation in some birds occurred in but a single test; in others the change was observed in two or more consecutive tests; while in others the titres varied at irregular test periods. Sustained trends in titre variations were not observed.

Obviously the number of birds showing variation in titres from that of non-reactor to reactor, would depend upon the titre limit accepted as indicating infection. Regardless of whether complete agglutination in the 1:25 or the 1:50 dilution be used as the minimum reactor titre, there were birds in each group that showed titre oscillations between reactor and non-reactor values.

SUMMARY

Adult hens showing different titre limits in agglutination tests for pullorum disease were assembled in two groups. In one group the birds were isolated in individual cages, in the other the birds were held as a unit in a single pen.

Agglutination tests were run at monthly intervals over a period of one year.

Titre fluctuations varying as to time of occurrence and degree were observed in each group.

There appeared to be no significant difference in sustained trends of titre variations of the two groups that could be attributed to the manner in which the fowls were maintained.

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The Collection of Spermatozoa from the Domestic Fowl and Turkey

W. H. Burrows and J. P. Quinn
National Agricultural Research Center, Beltsville, Maryland

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A NUMBER of methods for obtaining semen from the fowl have been described. Because of the peculiar genital anatomy of birds, the process of obtaining semen from them presents a somewhat different problem than that encountered in the mammal.

Ivanov (1913) obtained spermatozoa for experimental purposes by killing cocks, opening the abdomen and squeezing the semen from the vas deferens. Payne (1914), Craft, McElroy and Penquite (1926) and Jull and Quinn (1931) obtained spermatozoa by mating a cock to a hen and immediately securing the fluid from the cloaca of the hen; Payne, and Craft and his co-workers using a spoon and Jull and Quinn, a pipette. Amantea (1922) and Dunn (1927) placed a dish between "the two correspondents." Ishsikawa (1930) used an artificial cloaca consisting of an animal membrane bag on a wire frame. The artificial cloaca was attached to the hen and received the semen from the cock during treading. The semen was then removed to a suitable container. Adamstone and Card (1934) used a similar artificial cloaca, but substituted thin rubber for the animal membrane.

Burrows and Quinn (1935) briefly described a method for exciting the male fowl to a series of ejaculatory responses by a manual stimulation consisting of a rapid massage of the soft part of the abdomen just beneath the pelvic bones. When a fowl thus stimulated ejaculated, the semen was caught in a small beaker held beneath the vent.

This last-mentioned method has the advantage of giving the operator control over the time of collections, independent of the bird's desire to mate, and of allowing the collection of relatively large samples of semen. It also has some annoying disadvantages. The stimulation to the ejaculatory response also at times stimulates defecation, especially during the early training of the bird. Also reports show that some difficulty may be experienced in learning the technic.

This paper presents the results of the efforts to overcome these disadvantages.

ANATOMICAL

Contrary to popular belief, the rooster has a copulatory organ of appreciable dimensions. The so-called rudimentary copulatory organ is but the apex of the functional copulatory organ. The entire organ is located in the ventral wall of the cloaca and is normally concealed within the cloaca. Normal erection takes place at the time of copulation and persists for only one or two seconds, making observation of the organ difficult and a thorough examination impossible.

In carrying out this work it has been possible to maintain a condition of erection for several minutes. A clamping method was also devised so that the organ could be kept in a state of erection for detailed study after resection.

The so-called rudimentary copulatory

organ has been described as a ball of white gristly tissue that lies in the transverse folds of the cloaca. These transverse folds in the ventral wall of the cloaca are probably what is described by Kaupp as the "base of the papilla" which he says is "surrounded by a plexus of arteries and veins which serve as raises the base of the organ causing it to protrude still farther and gives the apex a downward thrust.

The organ when fully exposed is not unlike the semi-developed penis of the mammal or the overdeveloped clitoris of the mammalian female. It is roughly heart-

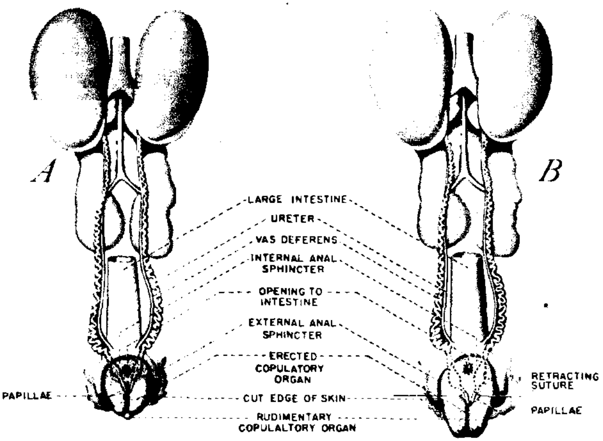

FIG. 1. The genital organs of the male fowl and the turkey. The bulbous ducts are shown between the *vasa deferentia* and the papillae by dotted lines.

an erectile organ during venereal orgasm." However, there are actually two areas of this erectable tissue; one on each side of the ventral midline. Because of the erection on each side, the ventral midline forms a furrow through which the semen flows. This furrow was also mentioned by Kaupp.

The erection of this tissue has the effect of straightening out the folds within the cloaca so that whereas it was longitudinally concave, it becomes convex. This change in form partially exposes the organ. The tightening of the internal sphincter of the anus shaped and varies in size from $\frac{1}{2}$mm. x $\frac{1}{2}$mm. to 2mm. x 2mm. See Figure 1-A.

The organ has no urethra through it, nor any connections whatsoever with the ureters, which enter the large intestine anteriorly to the internal sphincter of the anus and empty through two small papillae. It is, however, intimately associated with the openings of the vasa deferentia.

The vasa deferentia enter the dorsal wall of the large intestine in close proximation to the ureters, but they do not pierce the intestine at that point, as do the ureters.

The vasa deferentia proper empty into ducts which at that point lie between the walls of the large intestine, but which soon enter the musculature of the internal sphincter. These ducts traverse the dorsal quadrants of the sphincter, enlarge into bulbous pockets within the musculature and emerge from the ventral, posterior surface in two elongated papillae.

These papillae normally lie directly over the grove or furrow of the erected organ. The semen is ejected quite forcibly from these papillae during copulation, but generally follows the groove to the apex of the organ, which during copulation enters the protruded oviduct of the hen.

In the turkey cock the organ is very similar to that of the male fowl, except that it is larger and more blunt. Instead of the single apex it terminates in a double apex. See Figure 1-B.

The contraction that occurs in the internal sphincter of the anus at the time of orgasm appears to be the propelling mechanism which forces the semen contained within the bulbous part of the ducts out through the papillae. At the same time there appears to be considerable pressure within the abdomen from the straining, and this pressure forces semen from the vasa deferentia into the ducts as soon as the sphincter relaxes. If the semen is removed from the bulbous ducts before ejaculation, none will be ejected by an ejaculatory response, but after an ejaculatory response the ducts will be found to be filled as long as an adequate supply of semen is available.

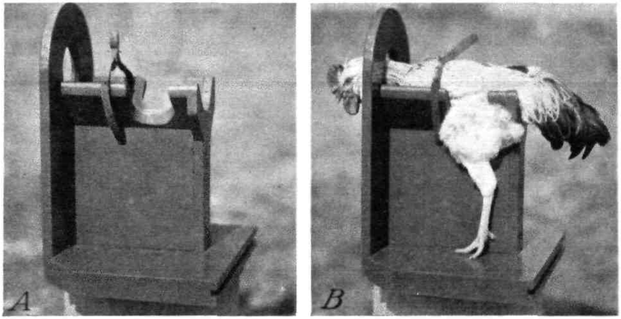

FIG. 2. A useful holder for birds when semen is to be collected by a single individual.

DETAILS OF THE METHOD

For convenience and economy of time the method herein described must be considered a two-man technic. However, since occasions do arise in which time is a less important factor than man-power, a holder for the bird has been devised. See Figure 2. It has been found that the use of the strap which is shown holding the wings in Figure 2-B, although quite convenient for obstreperous birds, can be dispensed with in most cases. The fact that the stand is high enough to prevent the bird from obtaining a footing on the base seems to discourage most attempts to escape.

The method is a modification of that described by Burrows and Quinn (1935).

FIG. 3. Showing the actual stimulation of the fowl. The operator's left hand is used here only to expose the field for the camera.

Originally a bird was stimulated to ejaculatory responses by massaging the soft sides of its abdomen between the gizzard and the pelvic bones. The copulatory organ, during these responses, erects, is thrust out and withdrawn with darting rapidity. The semen was caught in a small beaker which was held under the vent with the left hand. Figure 3 shows the position in which the bird is held, the manner of stimulation and the copulatory organ emerging from the vent.

It was noticed during the course of work with the original method that the stimulation could be augmented by laying the left hand on the bird's back and sliding the thumb and finger rearward about the pygostyle at the narrow place where it joins the body. It was but a step further to grip, with the thumb and forefinger, the base of the copulatory organ at the instant it was protruded, and to milk out the semen contained in the bulbous ducts. Later it was found to be more convenient in some instances to place the hand behind the tail when milking the ducts. See Figure 4-B.

Further experience showed that holding the organ exposed did not greatly interfere with the normal muscular contractions of the anal sphincter. Some birds will eject several copious spurts of semen by their own repeated muscular contractions if the operator simply holds the organ exposed as shown in Figure 4-B.

To make these modifications workable, the use of a beaker in the left hand to catch the semen had to be discontinued. A small 60-degree angle, glass funnel was fitted with

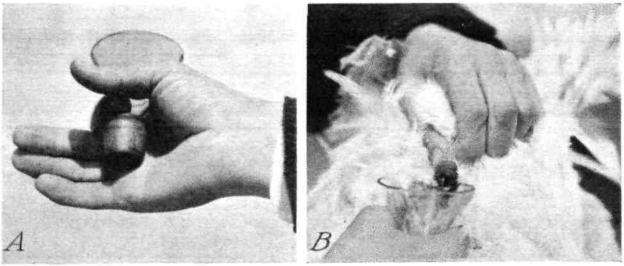

FIG. 4. (A) Shows the container and the manner of holding so that the fingers are free for stimulation purposes. (B) Shows the placement of the left thumb and forefinger in holding the copulatory organ exposed.

a one-hole, rubber stopper for a grip. That part of the stem below the stopper was cut off and the remaining stub was filled with paraffin. This container may be held with the right hand without interfering with the action of the fingers necessary for stimulating the bird. See Figure 4-A. The funnel has an added advantage in that the semen collects in a deep pool in the narrow bottom and can be more easily removed with a syringe.

To collect semen, the bird is slightly stimulated until the organ is protruded. The organ is held exposed with the left-hand fingers as long as the muscular contractions persist. Then the bulbous ducts are squeezed out and the organ is released. The container is tilted away from the vent and the bird is stimulated to make an ejaculatory response, at which no semen will be ejected if the ducts have been properly milked. It will usually be found that after the ejaculatory response the ducts are again full and the process can be repeated. It is possible to make from two to eight milkings at a single collection in this fashion, depending upon the bird and its condition. The amount of semen obtainable at one collection varies from 0.1 c.c. to as high as 4.0 c.c. in rare instances.

DISCUSSION

The method as herein described has been found to have several advantages over the original method described in 1935. The additional stimulation about the region of the tail and vent with the left hand aids greatly in causing some of the less responsive birds to undergo an ejaculatory response. Occasionally a bird is encountered which can be stimulated to an erection of the copulatory organ, but not to produce an ejaculatory response. Semen can be taken from these birds by simply milking the organ.

As suggested earlier in the paper, the semen can be milked into the container; and since the container can be removed during actual ejaculatory responses this practically avoids any soiling of the sample which is being collected.

Semen can be collected from turkeys by the milking process, whereas the original method was entirely unsatisfactory for use with turkey cocks.

Five turkey cocks have been used regularly from January until June to obtain semen for another investigation. They have remained in excellent condition. The average amount of semen obtainable from the turkey cock has been about 0.2 c.c.

A group of 22 crossbred male fowls, kept in batteries, have been under continuous experiment from November 8, 1935, until June 1, 1936. During this period of time collections were made at intervals of one, two, and three days; a total of 124 collections from each bird. Four of these birds became sick at one time or another during the experimental period and two of them died. This incidence of sickness and death was not in excess to what would normally be expected.

The birds in this experiment produced a total of 2,372 c.c. of semen. The best producer in the group yielded 203.8 c.c. and the poorest 44 c.c. As cockerels in November their individual output ranged from 0.1 c.c. to 1.1 c.c. of semen at a single collection. After December 1, 1935, and from then until the end of the experimental period, the range was from 0.4 c.c. to 3.6 c.c. In one exceptional instance the best producer yielded 4.0 c.c. at a single collection.

With the exceptions already noted, these birds remained in good health and apparently developed no abnormalities from the treatment.

It has been noted that one's first efforts to stimulate a male fowl to ejaculate are often attended with some difficulty. The birds vary greatly in responsiveness and it is obvious that learning would be much sim-

pler if the beginner used the more responsive birds. It is suggested that as large a number of birds as possible be utilized for the purpose of learning so that the chance of handling some of the more responsive birds may be greater.

After a little experience the operator seems to have no difficulty with any of the usual run of birds. All birds are more easily stimulated after they have been used for several collections.

Males running with hens usually yield very little, if any, semen. Segregation should be made at least two days before attempting to collect semen.

Males kept in individual pens or batteries apparently produce better than males running together. Males running together tread each other and fight, and in either instance there is a serious interference with the collection of semen.

SUMMARY

The posterior-most portions of the genital apparatus of the male fowl and the male turkey have been described in some detail and illustrated.

Modifications of the previously published method for obtaining spermatozoa from the fowl are described. These make possible greater ease of stimulation, less soiling of material obtained and the ability to milk semen from the ducts of fowls which cannot be stimulated to an ejaculatory response, and from male turkeys, which are very difficult to stimulate.

Some figures on collections are given which show that the method is satisfactory for both practical and experimental use.

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The Vitamin A Requirements of Chicks with Observations on the Comparative Efficiency of Carotene and Vitamin A*

P. R. RECORD, R. M. BETHKE, AND O. H. M. WILDER
Department of Animal Industry, Ohio Agricultural Experiment Station, Wooster, Ohio

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ALTHOUGH the literature contains numerous reports which show that the chicken requires vitamin A, there is little data available on the minimum requirements of this factor by the chick. Hauge, Carrick, and Prange (1926-1927) reported that 25 percent of yellow corn in the ration appeared to meet the vitamin A requirements of chicks to 10 weeks of age and that 50 percent of yellow corn appeared to supply sufficient vitamin A for the development of pullets to the laying stage. Smith (1933) also reported normal growth and viability in chicks up to 12 weeks of age when 25 percent or more of the total ration was made up of yellow corn. Kline, Schultze, and Hart (1932) found that 0.03 mg. of carotene daily was not sufficient as a sole source of vitamin A when chicks reached an age of seven to eight weeks. The same investigators reported that chicks depleted of vitamin A required more than 0.05 mg. of carotene daily to grow to maturity. Frohring and Weno (1934), from work on the carotene and vitamin A requirements of chicks, concluded that the daily minimal requirements at eight weeks of age was approximately 65 A.D.M.A. units per chick. These investigators also state that one microgram of carotene is equivalent to 2 A.D.M.A. units.

The object of the present studies was to obtain more information on the minimum requirements of chicks for vitamin A and to compare carotene and vitamin A from cod liver oil as sources of the vitamin. Several investigators (Capper, McKibbin, and Prentice, 1931; Kline, Schultze, and Hart, 1932; Frohring and Weno, 1934) have shown that the chicken can utilize carotene as a source of vitamin A, but no data pertaining to the comparative biological efficiency of carotene and vitamin A, as determined on rats, are available for the chicken.

EXPERIMENTAL

Day old White Leghorn chicks were used throughout. These were divided into groups and confined in brooders equipped with wire-grid floors. The same basal ration as described by Elvehjem and Neu (1932) was used in all trials. It consisted of white corn, 58.0; wheat middlings, 25.0; domestic casein, 12.0; dried yeast, 1.5; irradiated dried yeast, 0.5; steamed bone meal, 1.0; ground limestone, 1.0; and salt, 1.0. The irradiated yeast supplied 4,000 International units of vitamin D per 100 gm. of ration, which was ample to meet the antirachitic requirements of the chicken. Vitamin A was supplied either in the form of cod liver oil, carotene, or alfalfa leaf meal. The oil content of all rations was kept the

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same by diluting the cod liver oil and stock carotene solutions with cottonseed oil.

The experiments included a prophylactic type and a curative type of feeding. In the former trials, the vitamin A materials were incorporated in the basal ration every week to minimize the destruction of carotene or vitamin A. In the curative experiments, the chicks were fed the basal ration for 26 days before supplemental feeding was begun. At this time the vitamin A reserves of the livers had been depleted and the majority of the chicks showed definite vitamin A deficiency symptoms. The division of the chicks into groups was made on the basis of weight and sex. Measured amounts of the oil solutions were fed individually every other day. To minimize the loss of vitamin A and carotene, the different strength solutions were put in several small amber bottles and kept in the refrigerator. Each bottle contained enough solution for 10 to 12 days of feeding.

The products used as sources of vitamin A were fed to rats to determine their biological vitamin A equivalence in terms of the U.S.P. Reference cod liver oil. This procedure made it possible to not only express the unitage in terms of International units but also afforded a direct comparison of the two species with respect to their utilization of carotene and vitamin A.

The crystalline carotene used in the several experiments was supplied by the S.M.A. Corporation, Cleveland, Ohio. It represented a pure product prepared from carrots, which contained approximately 85 percent beta and 15 percent alpha carotene. The cod liver oil concentrate used in experiment 1 was furnished by the Health Products Corporation, Newark, N.J. The medicinal cod liver oil used in the curative experiment and the latter part of experiment 3 was purchased locally. The alfalfa leaf meal was a commercial sun-cured product obtained on the open market.

At the termination of the experiments the livers from 6 to 8 representative chicks from each lot were removed and assayed for blue units by the antimony trichloride (Carr-Price) color test, using the Lovibond tintometer. All determinations were made on pooled samples of the livers. In case of the chicks used in the curative experiments, the livers from 8 to 15 individuals, selected at random, were assayed for blue units at 1, 7, 14, and 21 days. Blue unit determinations were also made on pooled liver samples from day old chicks used in the prophylactic experiments. The chicks were also examined for the presence of urates in the internal organs and degenerative changes due to vitamin A deficiency at the time of death or upon termination of the experiments.

PROPHYLACTIC EXPERIMENTS

Two preliminary trials had indicated that under our experimental conditions it required somewhat more than 50 micrograms of carotene per 100 gm. of ration in the form of an impure preparation from carrots to meet the minimum vitamin A requirements of chicks to 8 weeks of age. These same trials suggested that the rat unit equivalent in the form of vitamin A from cod liver oil gave the same degree of protection in chicks as carotene. Accordingly, in the next trial (experiment 1) crystalline carotene dissolved in cottonseed oil and vitamin A, in the form of a cod liver oil concentrate, were each fed at three different levels. There was also included one group on the unsupplemented basal ration and a second group which received 2 percent of a poultry cod liver oil for negative and positive control purposes. The 2 percent of cod liver oil supplied approximately 1,500 units of vitamin A per 100 gm. of ration.

All lots grew normally up to the third week. At this time differences in the experimental lots became evident, both in weight and gross appearances. The chicks in lot 1, receiving the vitamin A-deficient basal ration, developed severe symptoms of

vitamin A deficiency during the fourth week and the majority of the birds died between the fourth and fifth weeks, with none surviving to the sixth week. All birds in lot 1, examined after death, showed an accumulation of urates in the ureters and kidneys. The chicks in lot 5 (25 units of cod liver oil) all developed external symptoms of vitamin A deficiency between the fourth and sixth weeks and all showed kidney or ureter lesions upon termination of the trial. Two chicks in this lot (lot 5) died of vitamin A deficiency between the fifth and seventh weeks. The other lots exhibited external and internal symptoms of avitaminosis in accordance with the amount of carotene or cod liver oil concentrate included in their ration. The results, presented in Table 1, show that it required between 50 and 100 micrograms of carotene and more than 75 units of vitamin A from cod liver oil per 100 gm. of basal ration to meet the minimum vitamin A needs of the chick to 8 weeks of age. The rat assay of the carotene and cod liver oil concentrate had shown that 50 micrograms of the carotene were equivalent to approximately 75 units of vitamin A. The results with chicks show that these same quantities per 100 gm. of ration gave about equal protection, indicating that the chick utilizes carotene as a source of vitamin A in the same order as the rat.

TABLE 1.—*The Carotene and Vitamin A Requirements of the Chick*

Lot No.	Vitamin A supplement per 100 grams basal ration	Weight at 8 weeks	Chicks showing vitamin A symptoms	Total* mortality	Blue units per gm. of liver
		gm.	percent	percent	
1	None	—	100	100	—
2	50 micrograms cryst. carotene	485 ± 10.3	15	0	0.0
3	100 micrograms cryst. carotene	554 ± 9.7	0	5	2.0
4	150 micrograms cryst. carotene	590 ± 10.6	0	20	3.6
5	25 units as cod liver oil concentrate	383 ± 16.1	100	20	0.0
6	50 units as cod liver oil concentrate	475 ± 16.3	45	10	0.0
7	75 units as cod liver oil concentrate	481 ± 9.8	10	0	0.0
8	2 percent cod liver oil (1500 units)	496 ± 12.5	0	10	174.0

* Includes all chicks that died.

In the next experiment we compared crystalline carotene, prepared from carrots, with alfalfa leaf meal as sources of vitamin A. Kane (1934) had reported that all of the vitamin A activity of alfalfa could be accounted for on the basis of its carotene content. The alfalfa leaf meal was analyzed for carotene and incorporated in the basal ration at different levels to furnish approximately the same amounts of carotene as were added in the crystalline form dissolved in cottonseed oil. The alfalfa leaf meal analyzed 0.0065 percent carotene and was fed at 0.8, 1.6, 2.5, and 5.0 percent levels. These amounts of alfalfa leaf meal supplied 52, 104, 162, and 325 micrograms of carotene per 100 gm. of ration, respectively.

The results, shown in Table 2, indicate that carotene either in the crystalline form or as found in alfalfa are about equally effective as sources of vitamin A for chicks. The blue unit values show somewhat more storage of vitamin A in the livers of the alfalfa leaf meal fed chicks than those birds which received approximately comparable amounts of crystalline carotene dissolved in oil. This suggests either that the chicks utilized the carotene from the alfalfa more efficiently than from the oil base or that there was a more rapid destruction of the carotene in oil than in the alfalfa leaf meal when incorporated in the basal ration. The results also show that 50 micrograms of

carotene per 100 gm. of basal ration was sufficient in this trial to meet the minimum vitamin A requirements of chicks to 8 weeks of age. The data, in this respect, appear to be inconsistent with the results of the previous experiments, in which case 50 micrograms of carotene per 100 gm. of ration were not sufficient to meet the vitamin A needs of the chick. It should be noted that this difference in results might be due either to the storage of vitamin A in the day-old chicks or the variation in the vitamin A content of the basal ration mixed at different times from different shipments of ingredients. It was noted in the last experiment that the chicks on the basal ration (lot 1) survived on the average from 7 to 10 days longer than in previous trials. The longer survival could be attributed to the basal ration or storage of vitamin A in the chick. It was observed that the livers from the day-old chicks in the last experiment assayed 67.0 blue units per gram of liver in comparison to 50.0 blue units for the liver from the chicks used in the earlier trials.

TABLE 2.—*The effectiveness of carotene and alfalfa leaf meal as sources of vitamin A*

Lot No.	Vitamin A supplement per 100 grams basal ration	Weight at 8 weeks	Chicks showing vitamin A symptoms	Total mortality	Blue units per gm. of liver
		gm.	percent	percent	
1	None	—	100	100	—
2	50 micrograms cryst. carotene	578±15.5	0	0	trace
3	100 micrograms cryst. carotene	617±13.2	0	0	2.2
4	150 micrograms cryst. carotene	541±11.6	0	0	3.8
5	52 micrograms carotene as alfalfa	579±10.3	0	0	2.0
6	104 micrograms carotene as alfalfa	567±11.7	0	0	4.6
7	162 micrograms carotene as alfalfa	595±10.2	0	0	16.5
8	325 micrograms carotene as alfalfa	565±12.5	0	0	43.0

Next we desired to obtain additional data on the comparative requirements and efficacy of carotene from alfalfa and vitamin A from cod liver oil for chicks to and beyond 8 weeks of age. The experimental procedure was the same as that of the previous trials, except that one-half of the cockerels in each lot were killed for examination and blue unit assay of the livers at 8 weeks and the remaining male survivors at 20 weeks. The pullets remaining at 20 weeks were continued on a laying experiment which will be reported at some future time. The alfalfa leaf meal was added to the basal ration so as to furnish approximately the same number of rat units per 100 gm. of ration, in the form of carotene, as were added in the form of cod liver oil. At the start of the experiment it was assumed, based on the report of the U. S. Pharmacopeia Vitamin Committee, that 0.6 micrograms of beta carotene was equivalent to 1 U.S.P. or International unit of vitamin A; which was later verified by rat assay of the alfalfa leaf meal in our laboratory. The U.S.P. Reference cod liver oil, containing 3,000 units of vitamin A per gm., was used for the first 6 weeks of the experiment. This was replaced with a medicinal cod liver oil which had been biologically assayed for vitamin A in comparison with the Reference cod liver both on rats and chicks. There was also included a positive control group which received the basal ration supplemented with one percent of a poultry cod liver oil having a potency of approximately 700 units of vitamin A per gm.

Table 3 gives a summary of the results at 8, 14, and 20 weeks. The weights at 8 weeks are an average of both sexes while the weights at 14 and 20 weeks are for pul-

TABLE 3.—*The comparative efficacy of carotene and vitamin A*

Lot No.	Vitamin A supplement per 100 grams basal ration	8 weeks				14 weeks		20 weeks			
		Weight*	Vitamin A symptoms	Total mortality	Blue units per gm. liver	Weight†	Vitamin A symptoms	Weight†	Vitamin A symptoms	Mortality‡	Blue units per gm. liver
		gm.	percent	percent		gm.	percent	gm.	percent	percent	
1	None	—	100	100	—	—	—	—	—	—	—
2	25 micrograms carotene as alfalfa	480 ± 9.5	68	0	0.0	760	100	804	100	33	0.0
3	50 micrograms carotene as alfalfa	545 ± 10.9	0	4	0.0	987	22	1224	44	17	trace
4	100 micrograms carotene as alfalfa	549 ± 11.6	0	8	trace	1128	0	1412	0	17	2.8
5	150 micrograms carotene as alfalaf	540 ± 10.8	0	4	2.0	1071	0	1401	0	17	8.4
6	200 micrograms carotene as alfalfa	552 ± 7.7	0	4	6.0	1113	0	1320	0	11	12.6
7	40 units as cod liver oil	504 ± 9.4	75	4	0.0	684	100	—	100	94	
8	80 units as cod liver oil	532 ± 8.6	4	0	0.0	893	61	951	89	44	
9	160 units as cod liver oil	564 ± 13.8	0	0	0.0	1091	0	1367	0	0	trace
10	240 units as cod liver oil	581 ± 13.0	0	4	1.4	1072	0	1337	0	6	6.0
11	320 units as cod liver oil	542 ± 9.9	0	4	6.4	1110	0	1376	0	0	7.5
12	1 percent poultry cod liver oil (700 units)	564 ± 7.7	0	0	59.8	1049	0	1307	0	0	155.0

* Average of male and female weights.
† Pullet weights only.
The mortality in Lots 4, 5, 6, 10, and 11 was from other causes than vitamin A.
‡ Mortality in this instance refers to all birds that died between the *8th* and *20th* week.

TABLE 4.—*Showing the decrease in the vitamin A content of the livers of chicks selected from the groups used in the curative experiments*

	Expt. 1	Expt. 2
Day-old	(15) 56.0	(15) 50.6
1 week	(10) 10.9	(10) 10.2
2 weeks	(8) 4.7	(10) 3.4
3 weeks	(8) 0.0	(10) 0.0

Figures in parenthesis refer to number of chicks killed for liver assay.

lets only. The cockerel weights were omitted at the latter periods, because several lots contained too few after the eighth week to give a fair average. The mortality and vitamin A deficiency data are based on both sexes, because no differences were noted between the response of the males and the females.

The results at 8 weeks (Table 3) show that 50 micrograms of carotene, in the form of alfalfa leaf meal per 100 gm. of basal ration or the rat unit equivalent (80 units) in the form of vitamin A from cod liver oil approximately met the minimum requirements of the chick to this age. The data also show that the vitamin A requirements of the birds increased with age, since part of the chicks on the 50 micrograms of carotene (lot 3) and 80 units cod liver oil (lot 8) levels showed definite symptoms of avitaminosis at 14 weeks, which increased in number and severity by the 20th week. The results indicate that, under the conditions of the experiment, the chicks required twice as much carotene (100 micrograms), or vitamin A (160 units) from cod liver oil per 100 gm. of ration to 20 weeks of age than to 8 weeks of age. In general there was no great difference in the response of the birds to carotene (alfalfa leaf meal) or vitamin A (cod liver oil) when equivalent rat units were fed; however, the data at 14 and 20 weeks suggest that the rat equivalent addition of vitamin A from cod liver oil did not give the same degree of protection as did the carotene. We are of the opinion that this probable difference is not due to the relative effectiveness of carotene and vitamin A but that there was a more rapid destruction of the vitamin A than the carotene in the ration, even though the feeds were mixed fresh at short intervals.

CURATIVE EXPERIMENTS

Two separate curative experiments were carried out. The first trial consisted of feeding different amounts of crystalline carotene dissolved in cottonseed oil to groups of 12 chicks each depleted of their vitamin A reserve (Table 4). The carotene solutions were fed every other day for a period of seven weeks. All the chicks in the control lot (lot 1) died of vitamin A deficiency on or before the 20th day after supplemental feeding was started. No mortality occurred in any of the carotene-fed lots except two chicks in lot 6 (200 micrograms carotene). One of these was killed accidentally and the second died from causes other than lack of vitamin A. The results in

TABLE 5.—*The effectiveness of carotene as a source of vitamin A for chicks (curative)*

Lot No.	Vitamin A supplement fed every other day	Average gain in weight	Chicks recovered*	Total mortality	Blue units per gm. of liver
		gm.	percent	percent	
1	None	47	0	100	—
2	20 micrograms cryst. carotene	437	17	0	0.0
3	40 micrograms cryst. carotene	535	67	0	0.0
4	60 micrograms cryst. carotene	567	92	0	0.0
5	100 micrograms cryst. carotene	619	100	0	trace
6	200 micrograms cryst. carotene	662	100	8	9.3

* The percentage of chicks that did not show external or internal vitamin A deficiency symptoms.

Table 5 show that it required between 60 and 100 micrograms of carotene every other day to cure and protect against avitaminosis.

The second experiment consisted of 12 lots of 10 chicks each depleted of vitamin A (Table 4). These were fed individually *every other day for 9 weeks different* amounts of crystalline carotene and rat vitamin A deficiency symptoms. The results with carotene are in agreement with the results obtained in the first curative trial. It should be noted that the above amounts of carotene and vitamin A did not allow for a storage of vitamin A in the livers of the chicks and in all probability represent the *absolute minimum daily requirements for* their age and the basal ration used.

TABLE 6.—*The comparative vitamin A and carotene requirements of chicks (curative)*

Lot No	Vitamin A supplement fed every other day	Average gain in weight	Chicks recovered*	Total mortality	Blue units per gm. of liver
		gm.	percent	percent	
1	40 micrograms cryst. carotene	595	10	0	0.0
2	60 micrograms cryst. carotene	629	40	0	0.0
3	100 micrograms cryst. carotene	719	100	0	0.0
4	200 micrograms cryst. carotene	733	100	0	9.3
5	40 units as Reference c.l.o.	469	0	0	0.0
6	80 units as Reference c.l.o.	615	33	0	0.0
7	120 units as Reference c.l.o.	715	80	0	0.0
8	200 units as Reference c.l.o.	795	100	10	0.0
9	40 units s medicinal c.l.o.	441	0	10	0.0
10	80 units as medicinal c.l.o.	635	50	0	0.0
11	120 units as medicinal c.l.o.	751	100	10	0.0
12	None	20	0	100	—

* The percentage of chicks that did not show external or internal vitamin A deficiency symptoms.

units of vitamin A from the U.S.P. Reference cod liver oil and a medicinal cod liver oil. The chicks that did not receive vitamin A (Lot 12) from some source all died of avitaminosis by the 21st day of supplemental feeding; whereas only 1 mortality (Lot 9) that could be attributed to vitamin A deficiency occurred in the remaining lots. The mortality in Lot 8 occurred the day after the experiment started and could not be attributed to experimental treatment and the death in Lot 11 was also from other causes than vitamin A.

From the data presented in Table 6, it is apparent that under the experimental conditions employed it required approximately 100 micrograms of carotene or between 120 and 200 rat units of vitamin A every other day to produce normal chicks and prevent

DISCUSSION

The results of both the prophylactic and curative experiments show that the chicken can use carotene as a source of vitamin A, which confirms the findings of Capper, McKibbin, and Prentice (1931), Kline, Schultze, and Hart (1932), Frohring and Weno (1934) and others. The fact that practically similar results were obtained with carotene (either prepared from carrots or as present in alfalfa) and cod liver oils when approximately equivalent rat units were fed, offers evidence that the chick and rat utilize carotene as a source of vitamin A in the same order.

The results of the curative experiments show that a minimum of 50 micrograms of carotene, or from 60 to 100 rat units of vitamin A were required daily to produce

normal chicks at 10 to 12 weeks of age. These observations are in agreement with those of Kline, Schultze, and Hart (1932) on carotene, and those of Frohring and Weno (1934) on carotene and vitamin A. Guilbert and Hart (1935) calculated, from the work of Guilbert and Hinshaw (1934), that the chicken required approximately 280 micrograms of carotene as alfalfa daily per kilogram of body weight. This amount of carotene caused a slight storage of vitamin A in the livers of the birds. In our experiments (curative) it required 200 micrograms of carotene in oil every other day to bring about a small storage of vitamin A in the livers of 10 to 12 week old chicks, weighing approximately 700 grams. Assuming that this level of carotene produced results comparable to those of Guilbert and Hinshaw (1934), our experiments indicate that the chicken requires about 150 micrograms of carotene per kilogram of body weight daily in contrast to 280 micrograms as calculated by Guilbert and Hart (1935).

The results of our prophylactic experiments are difficult to compare with the results of other investigators, because they either employed a different system of vitamin unitage or expressed their results in terms of percent of yellow corn. Fraps and Treichler (1933) reported that the vitamin A potency of yellow corn varied and that it lost some of its vitamin A value on storage. They stated that an average yellow corn when fresh contained about 5 Sherman-Munsell units of vitamin A per gram. Hauge, Carrick, and Prange (1926-1927) and Smith (1933) reported that 25 percent yellow corn in the ration appeared to meet the vitamin A requirements of chicks to 10 to 12 weeks of age. If we assume that the yellow corn these investigators used contained from 4 to 5 Sherman-Munsell units of vitamin A per gram, then the results of the above investigators would indicate that the chicks required from 100 to 125 Sherman-Munsell units per 100 gm. of ration. Sherwood and Fraps (1935) recently reported that chicks hatched from hens receiving an adequate supply of vitamin A required about 126 Sherman-Munsell units per 100 gm. of ration to reduce mortality to 12 weeks of age to near normal. The U. S. *Pharmacopeia Interim Revision of the* monograph for cod liver oil in 1934 states that 1 Sherman-Munsell unit of vitamin A is approximately equal to 1.4 International or U.S.P. units. On this basis, the work of the above investigators shows that about 140 to 175 International units of vitamin A per 100 gm. of feed were required by chicks —which is in fair agreement with the results of our prophylactic experiments.

Schroeder, Higgins, and Wilson (1935) in a recent study of the vitamin A requirements of chicks reported that chicks up to 9 weeks of age required 6000 International units of vitamin A per pound of feed (about 1320 units per 100 gm.) to prevent clinical and pathological symptoms of hypovitaminosis A. They also reported that 1200 units of vitamin A per pound of feed appeared to be adequate to promote fair growth; but that there was a tendency for body weights to increase as the vitamin A level was increased. The observations of these investigators are not in accord with the results of our studies, nor do they appear to be in agreement with the findings of Hauge et al (1926-1927), Smith (1933), Kline and associates (1932), and Frohring and Weno (1934). We have no explanation for these apparent discrepancies in results between different laboratories, unless the appearance of coryza and of fowl pox infections in the experiment of Schroeder and associates (1935) were contributing factors.

It should be noted that the optimum carotene or vitamin A requirements of growing chickens might be considerably higher than those (minimum) indicated by our tests. In this connection, both the pro-

phylactic and curative experiments showed that there was no significant storage of vitamin A in the livers of the chicks until several times the minimum level, as determined by growth and external and internal symptoms of avitaminosis, was fed. Green and Mellanby (1930) have reported that four times the minimum requirement of vitamin A were needed by rats to produce optimum results as far as resistance to infection was concerned.

Experiments are in progress to obtain further information on the minimum practical requirements.

SUMMARY

Experiments were conducted with White Leghorn chicks which included a prophylactic type and a curative type of feeding. The prophylactic trials showed that, under the experimental conditions employed, it required a minimum of approximately 50 to 100 micrograms of carotene or 80 to 160 International units of vitamin A from cod liver oil per 100 gm. of ration for normal growth and the prevention of external and internal symptoms of vitamin A deficiency in chicks to about 8 weeks of age.

The curative experiments showed that about 100 micrograms of carotene or 120 to 200 International units of vitamin A from cod liver oil were required every other day to cure and prevent symptoms of vitamin A deficiency and restore good growth in chicks depleted of their vitamin A reserve to 10 to 12 weeks of age.

Both types of experiments showed that the chick utilized carotene as a source of vitamin A. The response of the chicks to carotene or vitamin A was approximately similar when equivalent rat units were fed; indicating that the chick and rat utilize carotene as a source of vitamin A in the same order.

Data are presented which show that the vitamin A requirements of chicks increased with age.

It is pointed out that the minimum vitamin A requirements, as determined in these experiments, might not be the optimum requirements under practical conditions.

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The Vitamin A Requirements of White Leghorn Pullets During the Growing Period

GORDON E. BEARSE AND M. WAYNE MILLER
Department of Poultry Husbandry, Western Washington Experiment Station, State College of Washington, Puyallup

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THE apparent need for more study on the vitamin A requirements of chickens from date of hatch to maturity was responsible for the work herein reported being undertaken. Requirements studies by other workers have dealt in most instances with the first eight weeks of the chick's life.

Frohring and Wyeno (1934) found that the minimum requirements of vitamin A for White Leghorn chicks at the age of about eight weeks is approximately 65 A.D.M.A. units per day.

Record, Bethke, and Wilder (1935) report that in prophylactic trials from 50 to 100 gamma of carotene per 100 gm. of feed were required to produce normal growth and prevent symptoms of vitamin A deficiency. Fifty gamma of carotene were required daily in the curative test.

Sherwood and Fraps (1935) found that 150 Sherman-Munsell units per 100 gm. of feed gave optimum growth results with chicks for eight weeks.

Kline, Schultze, and Hart (1932) demonstrated that chicks that have been depleted of vitamin A require more than .05 mg. of carotene daily in order to grow to maturity. The estimation of vitamin A requirements by the daily feeding method is subject to error because of the great variation in size between a day-old chick and a mature pullet. A more logical approach to the problem would be to estimate the requirements either as number of units required per 100 gm. of feed or number of units required for 100 gm. of bird. It is evident that basing the requirement on the feed intake would be the simpler and more practical plan.

Throughout this paper references made to vitamin A refer to vitamin A activity regardless of whether its source is vitamin A, carotene or other vitamin A active substances.

EXPERIMENTAL

Six lots of 150 day-old Single Comb White Leghorn chicks were used in this study. A basal ration composed of 44.5 lbs. ground white corn, 15 lbs. ground wheat, 15 lbs. ground oats, 10 lbs. millrun, 7 lbs. meat scrap, 7 lbs. powdered skimmilk, 1 lb. oyster shell flour, and .5 lb. salt was fed to these birds. This ration analyzed 15.2 percent protein, 1.14 percent calcium, .75 percent phosphorus and 3.6 percent fiber. Dehydrated alfalfa was used as a source of vitamin A to supplement this ration as follows:

Lot 1. Control. No supplement. 100 lbs. basal mash.

Lot 2. 0.5 lb. dehydrated alfalfa and 99.5 lbs. basal mash.

Lot 3. 1.0 lb. dehydrated alfalfa and 99.0 lbs. basal mash.

Lot 4. 2.0 lbs. dehydrated alfalfa and 98.0 lbs. basal mash.

Lot 5. 4.0 lbs. dehydrated alfalfa and 96.0 lbs. basal mash.

Lot 6. 8.0 lbs. dehydrated alfalfa and 92.0 lbs. basal mash.

The dehydrated alfalfa analyzed 20.0 percent protein, 18.3 percent fiber, 1.53 percent calcium, and .22 percent phosphorus.

Vitamin D was supplied by irradiating the birds with a Cooper-Hewitt quartz mercury lamp for 10 minutes each day. This was not used on day when sunlight irradiation was possible.

The units of vitamin A in the dehydrated alfalfa were determined with rats by the method of Sherman and Munsell (1925). Details of the vitamin A assay are shown in Table 1. These results show that the dehydrated alfalfa contained 200 Sherman-Munsell units in that lot 6 averaged slightly more than a unit gain per week when fed at a level to test for 200 units. Sherman and Munsell (1925) define a unit as that amount of vitamin A which, when fed daily, induces an average gain of 3 gm. per week under the conditions they describe.

TABLE 1.—*Summary of vitamin A assay of dehydrated alfalfa, March 21, 1934 to May 28, 1934*

Lot No.	No. rats	Mg. fed daily	Ave. initial weight	Ave. final weight	Ave. gain	Remarks
		mg.	gm.	gm.	gm.	
1	9	basal	121.9	123.3	1.4	2 rats survived eight-week period
2	7	40.00	118.0	237.8	119.8	
3	6	20.00	110.7	213.7	103.0	
4	9	10.00	103.1	171.8	68.7	
5	8	7.50	111.4	154.6	43.2	
6	7	5.00	109.4	147.6	38.2	
7	5	3.75	113.4	133.2	19.8	

On the basis of 200 units of vitamin A per gram of dehydrated alfalfa the lot 2 ration would supply 100 units; lot 3, 200 units; lot 4, 400 units; lot 5, 800 units; and lot 6, 1,600 units per 100 gm. of feed.

A second assay was conducted at the end of the trial in which the same dehydrated alfalfa was tested at levels of 150 and 200 units. The rats on the 200-unit level did not make unit gains. Six rats completing the trial on the 150-unit level for the five-week period made an average gain of 13 gm. As this is practically a unit gain it is assumed that the dehydrated alfalfa at the end of the trial assayed approximately 150 units. Thus, the levels indicated above apply only to the alfalfa at the beginning of the experiment. The average number of vitamin A units fed to each lot based on the average of the two assays is shown in Table 2.

The chicks were wingbanded and brooded for the first two weeks in electric brood units composed of compartments 74½ x 26½ x 8½ in., decked one on the other. Seventy-five chicks were placed in each compartment. These brooders were located in the nutritional laboratory of the poultry department in a room held at a temperature of approximately 70°F.

After two weeks in the battery brooders the chicks were transferred to pens 12 ft. x 18 ft. in a brooder-house and brooded under radiant heat electric brooders until they were perched. Cockerels were removed from the age of four weeks on as soon as they could be detected. The birds had access to a sunporch 6 ft. wide at all times from three weeks of age to maturity and at no time were allowed on the ground. Litter was changed three times a week throughout the experiment to reduce the danger of parasitic infestation.

The feed ingredients were stored in a feed room and the rations mixed at approximately 10-day intervals. The ration was kept before the birds in hoppers at all times. Grit was supplied ad libitum but no

TABLE 2.—*Summary of results. The vitamin A requirements of White Leghorn pullets during the growing period*

Lot No.	Supplement fed	Ave. No. Sherman units per 100 grams ration	No. pullets started	No. pullets dying from avitaminosis A	Total No. pullets died	Percent mortality	Ave. weight of pullets		Grams feed per gram grain		Total feed consumed	Vitamin A units in liver (3 birds)
							8 weeks	24 weeks	8 weeks	24 weeks		
							gm.	gm.	gm.	gm.	gm.	
1	None	—	81	63	78	96.3	291.1	796.0	5.32	7.57	12.57	0
2	½ per cent dehydrated alfalfa	87.5	66	9	20	30.3	425.8	1152.0	4.22	6.73	16.57	0
3	1 per cent dehydrated alfalfa	175.0	79	0	6	7.6	453.9	1353.2	3.91	6.32	18.33	6.66
4	2 per cent dehydrated alfalfa	350.0	87	0	12	13.8	461.7	1333.9	3.78	6.39	18.38	1109.00
5	4 per cent dehydrated alfalfa	700.0	75	0	4	5.3	488.4	1409.7	3.52	6.30	19.11	4124.60
6	8 per cent dehydrated alfalfa	1400.0	70	0	2	2.9	487.8	1430.1	3.78	6.35	19.58	15930.00

other supplements to the rations were fed.

Feed and individual body weight records were made every two weeks. An accurate record of mortality was kept and post-mortem examinations made of all dead birds.

The duration of the experiment was 24 weeks commencing April 16, 1934. At the end of the experiment three representative birds were selected from each lot and the livers removed for determination of their vitamin A units. The method of Guilbert and Hart (1934) was used in these determinations. Inasmuch as the birds reared in this experiment were to be used for egg production studies it was not advisable to sacrifice more of them for this purpose.

RESULTS AND DISCUSSION

The results of this experiment are summarized in Table 2. It is evident from this table that the basal ration used is very deficient in vitamin A as there were only three birds remaining in lot 1 at the end of the trial.

The mortality declines from lot to lot as the level of vitamin A increases. None of this mortality was diagnosed as vitamin A deficiency in lots 3, 4, 5, and 6. Six of the nine birds that died from vitamin A deficiency in lot 2 died during the last six weeks of the experiment. Since the mortality was higher from other causes in those lots receiving the lower levels of vitamin A it would seem desirable to include large amounts of this vitamin in the growing ration.

The birds on the 175 Sherman unit level made approximately the same gains in weight as the birds receiving the higher levels. Inasmuch as there was no mortality in this lot diagnosed as avitaminosis A, it would seem that the vitamin A requirements have been met and that the slightly higher weight figures for lots 5 and 6 might have been due to slight differences in other nutrients supplied by the addition of the dehydrated alfalfa.

Hauge, Carrick, and Prange (1927) found that 25 percent yellow corn supplied in the ration met the vitamin A requirements of chicks for the first 10 weeks of their lives. This would approximate 160 Sherman-Munsell units of vitamin A per 100 gm. of feed based on 6.4 Sherman-Munsell units per gm. of yellow corn which is the average assay reported by Fraps (1931) for the yellow corn varieties tested.

Feed efficiency was greater in the lots receiving the highest levels of vitamin A during the first eight weeks of the trial. This difference was not so great for the entire 24-week period. There is a close correlation between mature body weight and total feed consumption.

Table 2 shows that there was no storage of vitamin A in the livers of the birds examined from lots 1 and 2 and very little from lot 3. However, the fact that there was some storage in the livers of three birds examined in lot 3 would indicate that the vitamin A requirements for growth had been met.

SUMMARY

Day-old Single Comb White Leghorn pullets fed a vitamin A deficient basal ration were supplemented with varying levels of dehydrated alfalfa for a 24-week growing period. These levels furnished approximately 87.5, 175, 350, 700, and 1,400 Sherman-Munsell vitamin A units per 100 gm. of ration.

The results based on avitaminosis A mortality, growth and liver storage of vitamin A show that 175 Sherman-Munsell vitamin A units per 100 gm. of ration met the vitamin A requirements of growing White Leghorn pullets under the conditions of this experiment.

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The Effect of Varying Levels of Vitamin A in the Hen Ration on the Vitamin A Content of the Egg Yolk, on Hatchability and on Chick Livability

Gordon E. Bearse and M. Wayne Miller
Department of Poultry Husbandry, Western Washington Experiment Station, State College of Washington, Puyallup

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POULTRY producers have long considered it desirable to include large amount of green feed in the ration of breeding hens. Since the discovery and development of our knowledge concerning vitamin A it has been assumed that this green feed was fed to supply large amounts of this vitamin and that vitamin A was closely associated with hatchability and chick livability. Actual corroboration of this assumption by experimental data is limited.

Throughout this paper references made to vitamin A refer to vitamin A activity regardless of whether its source is vitamin A, carotene or other vitamin A active substances.

Sherwood and Fraps (1934) found that the hatchability increased as the level of heat-dried alfalfa leaf meal increased up to 8 percent of the mash. Later work by these authors (1935) did not show an increase in hatchability for an 8 percent alfalfa group over a 4 percent one. Studies reported by Payne and Hughes (1933) indicate that the addition of green feed to poultry rations increased their hatchability.

Several workers including Sherwood and Fraps (1932, 1934 and 1935), Bisbey et al. (1934), DeVaney, et al. (1935), and Bethke et al. (1927) have demonstrated the relationship between the vitamin A content of the ration and the vitamin A content of the egg yolk by means of vitamin A assays of egg yolk.

In view of the above fact it would be expected that chicks hatched from eggs containing various quantities of vitamin A would contain various amounts of this vitamin in their bodies. The greater the potency of the egg yolk the larger the amount possessed by the chick at hatching. Chicks fortified with large quantities of vitamin A in their bodies should therefore survive longer on a vitamin A deficient diet than chicks which had acquired very little of this vitamin at hatching. Work by Sherwood and Fraps (1935) indicates that this is true.

EXPERIMENTAL

The Single Comb White Leghorn hens used in this experiment were raised on the rations outlined by Bearse and Miller (1936). At the start of the laying period there were 65 birds in each pen with the exception of lot 2 which had only 46. During the laying period the hens were fed a basal ration composed of 41.5 lbs. ground white corn, 15 lbs. ground wheat, 15 lbs. ground oats, 10 lbs. millrun, 7 lbs. meat scrap, 7 lbs. powdered skimmilk, 2.5 lbs.

TABLE 1.—*Summary of first vitamin A assay of egg yolks started October 21, 1934. Sherman-Munsell method*

Lot No.	Units per 100 grams of hen ration	No. rats beginning	No. rats end	Mg. yolk fed daily	Ave. initial weight	Ave. gain in 8 weeks	Sherman unit level	Remarks
				mg.	gm.	gm.		
1	150	6	6	200.0	105.2	50.00	5	
2	150	6	4	100.0	104.7	12.20	10	
3	300	6	6	200.0	97.0	91.80	5	
4	300	6	4	66.7	105.2	36.00	15	
5	600	6	5	50.0	98.7	44.20	20	
6	600	7	4	25.0	104.4	13.25	40	
7	1200	6	6	50.0	102.2	55.50	20	
8	1200	6	5	25.0	101.2	26.00	40	
9	—	7	0	—	96.7	0.00	0	Basal

oyster shell flour, 1.5 lbs. bone meal and .5 lb. salt. This ration analyzed 14.8 percent protein, 2.06 percent calcium, .90 percent phosphorus, and 3.6 percent fiber. Dehydrated alfalfa was used as a source of vitamin A to supplement this ration, as follows:

Lot 2. 0.5 lb. dehydrated alfalfa and 99.5 lbs. basal mash.

Lot 3. 1.0 lb. dehydrated alfalfa and 99.0 lbs. basal mash.

Lot 4. 2.0 lbs. dehydrated alfalfa and 98.0 lbs. basal mash.

Lot 5. 4.0 lbs. dehydrated alfalfa and 96.0 lbs. basal mash.

Lot 6. 8.0 lbs. dehydrated alfalfa and 92.0 lbs. basal mash.

The dehydrated alfalfa analyzed 20.0 percent protein, 18.3 percent fiber, 1.53 percent calcium, and .22 percent phosphorus. The vitamin A content of the alfalfa was determined by the Sherman-Munsell (1925) method and was found to contain approximately 150 units at the time the first egg yolk assay was made. Later in the laying period at the time the hatching studies and second egg yolk assay were made, the alfalfa was again assayed and found to contain 125 Sherman-Munsell vitamin A units.

The ration was fed as an all-mash and kept before the birds at all times. Grit was supplied ad libitum. No other supplements were fed.

Representative samples of egg yolk from each lot were assayed for their vitamin A potency by the Sherman-Munsell (1925)

TABLE 2.—*Summary of second vitamin A assay of egg yolks started May 2, 1935. U.S.P.X. 1934 method*

Lot No.	Units per 100 grams of hen ration	No. rats beginning	No. rats end	Mg. yolk fed daily	Ave. initial weight	Ave. gain in 4 weeks	U.S.P. unit level	Remarks
				mg.	gm.	gm.		
1	—	7	0	—	65.7	0.0	0	Basal
2	—	7	6	.7*	63.7	45.5	3000	Reference
3	250	7	6	300.0	63.7	66.6	7	
4	250	7	7	150.0	68.1	26.2	14	
5	500	7	6	150.0	64.0	55.5	14	
6	500	7	7	75.0	65.3	17.0	28	
7	1000	7	6	75.0	63.7	37.7	28	
8	1000	7	6	50.0	64.3	17.5	42	

* .7 mg. U.S.P.X. 1934 reference cod liver oil.

TABLE 3.—*Vitamin A potencies of egg yolk from rations of various vitamin A potency as determined by the assays shown in Tables 1 and 2*

Hen Lot No.	First assay—October 21, 1934		Second assay—May 2, 1935		
	Sherman units per 100 grams of hen ration	Sherman units in egg yolk	Sherman units per 100 grams of hen ration	U.S.P. units in egg yolk	Sherman unit equivalent
3	150	7.5	—	—	—
4	300	20.0	250	10.5	7.5
5	600	30.0	500	21.0	15.0
6	1200	40.0	1000	28.0	20.0

method at the beginning of the laying period. After the laying period had progressed six months a second assay was conducted using the U.S.P.X. 1934 method. This change in technic was made to increase the accuracy of the vitamin A assays. Twelve eggs, or the entire day's production when less than 12 eggs, were taken from each pen bi-weekly for supplementing the diet of the rats. The yolk was removed from the albumen so that none adhered to it. The yolks were then thoroughly mixed together and a weighed quantity was in corporated into the vitamin A free basal rat diet by grinding in a mortar. The mixture was placed in an air-tight glass jar and kept in an electric refrigerator at a temperature of approximately 36°F. A weighed sample of this mixture was used to supplement the diet of the rats.

For the hatchability studies four cockerels were placed in each pen and moved to a new pen three times a week. Eggs were saved periodically during the months of March and April and incubated in the same electric incubator.

The chicks from four hatches were individually banded and placed in electric brood units. They were fed the vitamin A deficient basal ration described by Bearse and Miller (1936). Vitamin D was supplied by irradiation. Individual weights were recorded weekly until death occurred. An accurate mortality record was kept.

RESULTS AND DISCUSSION

Table 1 summarizes the details of the vitamin A assay of egg yolks conducted shortly after the birds came into production. In addition to the fact that the hens' rations supplied various levels of vitamin A, the birds on the higher levels had greater body storage of vitamin A at the beginning of the experiment because of the effect of their growing rations.

The details of the assay of the vitamin A potency of egg yolks made after the birds had been in production for several months is shown in Table 2 and the results summarized in Table 3. The lower unit values for the egg yolks assayed after the hens had been in production several months can hardly be accounted for by the decrease in vitamin A potency of the hens' rations. It

TABLE 4.—*A summary of the nine weekly hatches of the eggs from the hens of the five lots*

Lot No.	Per cent of alfalfa in hen ration	Sherman units per 100 grams of hen ration	No. eggs set	Per cent fertility	Per cent hatch of fertile eggs
2	0.5	62.5	2	100.0	0.0
3	1.0	125.0	67	89.6	68.3
4	2.0	250.0	113	85.8	84.5
5	4.0	500.0	240	88.8	95.8
6	8.0	1000.0	299	89.3	89.1

would seem that there must have been considerable depletion of the hen's body storage of vitamin A.

Table 4 is the summary of the nine weekly hatches for the five lots of hens. The differences in numbers of eggs set were due

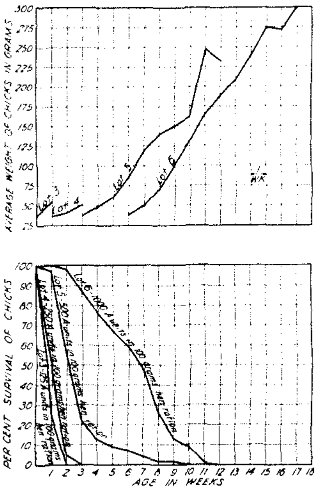

FIG. 1. Curves showing growth and per cent alive of chicks fed a vitamin A deficient ration from *hens fed varying levels of vitamin A.*

to differences in production in the pens. The hatchability of the eggs increased as the level of vitamin A in the ration was increased up to lot 5 inclusive. Lot 6 did not give a further increase in hatchability. Postmortem records of the hens dying in this experiment showed that avitaminosis A occurred in lots 2, 3, and 4. Since these lots were deficient and were the only ones in which an impairment of hatchability occurred it is evident that when enough vitamin A is present to prevent mortality from this cause there is sufficient for hatchability. Although we are not concerned with egg production in the work herein reported, yet it should be mentioned that the production increased as the level of alfalfa was increased in the ration; thus, it appears that a ration containing sufficient vitamin A for maximum hatchability may not contain sufficient for maximum production.

In contrast to this Carver et al. (1934) *reporting on vitamin D requirements of* chickens stated that 135 international units of vitamin D per 100 gm. of feed were required for satisfactory hatchability, whereas only 67 units were required for satisfactory production.

Chick livability and growth on a vitamin A deficient diet were greatly influenced by the quantities of vitamin A in the hen ration. This is illustrated by Figure 1. The four chicks from lot 3 survived an average of 7.3 days on this ration. The period of survival for the 19 chicks in lot 4 was 8.3 days; for the 68 chicks in lot 5 it was 19.2 days; and for the 92 chicks in lot 6 it was 44.7 days. The increased growth and the lengthened survival period of the chicks from the higher vitamin A level breeders' rations show that the vitamin A content of the egg yolk is responsible for the vitamin A content of the chick's body at hatching time. It serves to supply the chick with vitamin A which insures growth and livability during the initial period of its life.

SUMMARY

Eggs from hens receiving different quantities of vitamin A in their rations contained different quantities of vitamin A in their egg yolks in proportion to the amount of vitamin A in the ration.

Chicks hatched from such eggs lived and grew on a vitamin A deficient ration in proportion to the amount of vitamin A in the breeding hen ration.

Five hundred Sherman-Munsell vitamin

A units per 100 gm. of feed in the breeding hen ration supplied sufficient vitamin A for maximum hatchability.

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News and Notes

Annual Meeting.—Mark your new calendars now for Poultry Science annual meeting to be held at Madison, Wisconsin, Aug. 10-13, 1937.

Dr. Walter Landauer, Agricultural Experiment station, Storrs, Connecticut, is anxious to obtain newly hatched chicks with posterior duplication (four legs). Even more desirable for his purposes are the parents of such chicks.

H. M. Scott, Associate Professor of Poultry Husbandry, Kansas State College, is on sabbatic leave this year for the purpose of continuing his graduate work at the University of Illinois. Robert E. Phillips (M.S., Kansas, 1936) is substituting for Professor Scott.

The new 20 x 100 foot brooder house completed last February at Kansas State College was destroyed by fire November 29. The loss exceeded $2000. Authorization was given by the college officials to replace the building at once.

Genetical Differences in Eight-Week Weight and Feathering

R. GEORGE JAAP AND LAWRENCE MORRIS
Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma

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FROM a broiler production standpoint it is important that breeds and varieties of chickens grow and feather rapidly during the first two or three months of their life. Since it is only the hereditary differences which make possible any improvement in the varieties, it is important to know the extent to which differences in early growth and feathering are caused by environmental factors and how much they are caused by heredity. The present study was undertaken to measure the relative importance of some of the chief causes of variation when the chickens are fed and managed in the same manner during the same year.

SOURCE OF DATA

The records analyzed in this report were obtained during the early part of the 1936 brooding season. They consist of weight and feathering records of pedigreed eight-week-old chickens from six standard varieties and similar records from a crossbreeding experiment. Since these chickens were all hatched within three months (January 18, to March 15), fed and managed in the same manner, they represent a population of individuals in which variation due to different environmental conditions is considerably lower than that of different flocks or of the same flock in different years.

The weight records are given in pounds, readings being taken to the nearest 0.05 lb. For feathering, an arbitrary classification is chosen using the values 1, 2, and 3. This classification is based primarily on the condition of the back and wings. A value of 1 designates a chicken whose back and wing fronts either lack feathers or have only a few scattered pinfeathers showing. Number 2 includes those chickens with a relatively large number of pinfeathers in these sections, indicating that they would probably have a good complement of feathers within approximately one month's time. A chicken designated as 3 has its full complement of feathers. This classification is somewhat broader than that used by other investigators (cf. Gericke and Platt, 1932) but has served its purpose in portraying the "degree" of feathering in each of the classes subsequently analyzed. The records are analyzed by the method of analysis of variance and covariance described by Fisher (1934) and Snedecor (1934). All differences discussed in this paper are highly significant (P. .01) and all figures following the ± sign are the standard errors.

GROWTH

The mean eight-week weight for each of the six varieties is presented in Table 1. These records differ from previous findings in the relation between the heavier varieties, for example, Barred Rocks, and the lighter weight variety, Leghorns. Previous researches have indicated that those varieties which are heavier as adults, although they may have a longer growing period, have a more rapid early growth and a resultant heavier weight at eight weeks of age. Warren (1930), Waters (1931), Lerner and Asmundson (1932), and Asmundson and Lerner (1934) show a greater eight-week

TABLE 1.—*Eight-week weight and "degree" of feathering for six standard varieties*

Variety	No. of individuals	Mean 8-wk. weight lb.	Mean degree 8-wk. feathering	Mean adult pullet weight lb.
Buff Orpington	241	1.396 ± .015	2.9 ± .045	6.0
White Wyandotte	99	1.243 ± .024	1.8 ± .070	5.2
Rhode Island Red	232	1.227 ± .016	2.0 ± .046	5.7
White Leghorn	543	1.217 ± .010	(3.0)	3.5
Barred Plymouth Rock	157	1.124 ± .019	2.3 ± .056	5.2
White Plymouth Rock	185	1.071 ± .018	1.9 ± .051	5.5

weight for Barred Rocks, Reds, Black Giants, Light Sussex, and Brahmas than that of the White Leghorn or Mottled Ancona. Asmundson and Lerner (1934) and Schnetzler (1936) have found that the growth rate to eight weeks of age is related to the final weight in Barred Rocks and White Leghorns. Separate figures obtained for some of these breeds by other investigators tend to indicate similar results. In the varieties considered here this tendency is not true. The Leghorns tend to be intermediate in the weight range of the varieties observed. For this reason it was thought desirable to include in Table 1 the mean adult pullet weight obtained in the fall of 1935 from unselected individuals of these varieties. These data indicate that early growth, at least to eight weeks of age, is a separate entity and not necessarily associated with mature weight. This does not preclude the possibility of an intra-varietal relation between eight-week and adult weight.

TABLE 2.—*Influence of sire on eight-week weight and feathering*

Variety	Sire	No. of progeny	Mean weight *lbs.*	Mean feathering
Buff Orpington	A	111	1.31	2.9
	B	130	1.47	3.0
White Wyandotte	A	27	1.28	1.7
	B	44	1.24	1.7
	C	9	1.26	2.2
	D	19	1.18	1.8
Rhode Island Red	A	87	1.23	2.0
	B	54	1.01	1.5
	C	91	1.35	2.2
White Leghorn	A	74	1.25	
	B	57	1.19	
	C	198	1.19	
	D	87	1.31	
	E	79	1.13	
	F	48	1.27	
Barred Plymouth Rock	A	14	1.16	2.5
	B	76	1.09	2.1
	C	67	1.16	2.5
White Plymouth Rock	A	39	1.06	1.9
	B	27	1.07	2.3
	C	119	1.08	1.8

Within each variety the progeny of individual males and of individual females differ significantly. The mean weights for the progeny of different males are given in Table 2. Since that for individual female parents is of the same order their means are not presented here.

Sex has a greater influence on the early growth of some varieties than on that of others. The influence of sex upon the progeny of different varieties is presented in Table 3. Asmundson and Lerner (1933) suggest that the difference between the early growth rate of the sexes is greater when the growth of both sexes is more rapid. Such a relation does not exist in the data presented in Table 3.

TABLE 3.—*Summary of eight-week weight differences due to sex*
Eight-week weight in pounds

Variety	Progeny	Male	Female	Difference	Diff. in percent of females, weight
Barred Plymouth Rock	157	1.164	1.083	$.081 \pm .034$	7.5
White Leghorn	543	1.321	1.118	$.203 \pm .018$	18.1
*White Plymouth Rock	73	1.184	1.038	$.146 \pm .050$	14.0
*Buff Orpington	93	1.318	1.226	$.092 \pm .044$	7.5
Cross (B.O.XW.R.)	244	1.411	1.242	$.169 \pm .027$	13.6
Cross (W.R.XB.O.)	89	1.336	1.228	$.108 \pm .044$	8.8
Total	1199	1.312	1.150	$.162 \pm .014$	14.1

* The White Rocks and Buff Orpingtons considered here are from different populations than those included in previous tables.

Standard deviations within variety, sire, dam and, sex for this material are: Entire population, 0.26; within varieties, 0.24; from each male, 0.21; progeny of the same hen, 0.17; and individuals of the same sex and variety, 0.21. The consistency of the different groups of progeny is measured by the intra-class correlation coefficients which are 0.22 between weights chosen at random from the offspring of the same male when mated within the variety and 0.39 between offspring of the same hen when mated to one male of the same variety. Progenies of the same sire or the same dam are, therefore, distinct groups with a certain uniformity obtained from the influence of the parent. Uniformity of the progeny of a sire is probably accentuated by consanquinity of the hens to which he is mated.

When conditions of feeding and management are identical and the chicks are produced within a relatively short period, variety, sire, dam, and sex account for 82 percent of the variance. The degree to which each of these factors influence growth to eight weeks of age may be fairly accurately measured. Variety accounts for 13 percent; sire, 19 percent; dam, 27 percent; and sex, 23 percent of the total variance. The residual error amounting to 18 percent of the total variance is caused by numerous factors. Size of egg, time hatched, physiological differences due to environmental response, and many other factors contribute to this portion of the variance. Each of these probably have only a small effect.

Since Upp (1928) and Funk, *et al.* (1929) were unable to demonstrate any relation between the weight of egg and subsequent growth of the chick, egg weight has probably a minor influence on the residual error. Asmundson and Lerner, in one case (1933), obtained a significant difference in growth rate between early and late hatches. In the present data, smaller groups within a variety which were adequately represented in each hatch did not indicate significant differences. It is probable, therefore, that time of hatch gives only a small contribution

to the total variance. For both eight-week weight and feathering it is assumed that physiological differences in response to environment account for the major part of the residual error.

FEATHERING

Tables 1, 2, and 4 include some of the more essential data on eight-week feathering. In estimating the influence of variety, sire, dam, and sex on feathering the data from the White Leghorns and one strain of Buff Orpingtons are omitted. These two varieties were practically all well-feathered at eight weeks of age. The percentage of the total variance for feathering due to differences of varieties is 4; of sires, 11; of dam, 16; and sex, 20. Together they account for 51 percent of the total variance. It is evident that when there are both poor and well feathered individuals in a population at eight weeks of age, 49 percent of this variability is not inherited. Therefore, one would expect to make much more rapid progress selecting for weight than for feathering from observations taken the eighth week after hatching.

TABLE 4.—*Influence of sex on feathering at eight weeks*

Variety	Number		Degree Feathered		Diff. Standard Error
	Males	Females	Males	Females	
Barred Rock	81	76	2.01	2.62	0.61 ± 0.105
White Rock	27	36	1.59	2.39	0.80 ± 0.166
Buff Orpington	42	51	2.45	2.73	0.28 ± 0.136
Buff Orpington × White Rock	124	114	2.06	2.76	0.70 ± 0.085
White Rock × Buff Orpington	28	34	2.00	2.38	0.38 ± 0.167
Total	302	311	2.05	2.64	0.59 ± 0.03

Warren (Payne and Scott, 1934), by use of the sex-linked gene for early tail and wing feathering, has been able to produce a strain of Rhode Island Reds which "feather out early like Leghorns." While this may be the simplest solution to the feathering problem, it may be interesting to note that the rapid feathering Buff Orpingtons previously discussed contain very few, if any, sex-linked rapid feathering individuals.

COVARIANCE FOR EIGHT-WEEK WEIGHT AND FEATHERING

Many investigators have been led to believe that the more rapid growing individuals feather better than the slower growing ones. High correlation coefficients have been obtained, for example, by Gericke and Platt (1932). They found a correlation of $+.8120 \pm .109$ between weight and feathering at eight weeks of age. The Barred Rocks used by these investigators, however, were fed and managed in several different ways.

The records of five groups for which both eight-week weight and feathering are available are analyzed for covariance. These five groups are (1) Barred Rock, (2) Buff Orpington, (3) White Rock, (4) progeny of Buff Orpington male X White Rock females, and (5) the reciprocal of (4). The crossbreds are half-brothers and half-sisters to the Buff Orpingtons and White Rocks. Correlation coefficients of 0.62 for weight and feathering of the groups and −0.28 for between sexes may easily have arisen from accidents of sampling in a non-correlated population. The coefficient obtained for the population as a whole (0.23), and the correlations obtained for within groups (0.20) and for between chicks of the same sex within the groups (0.33) are significant. The coefficient of determination as estimat-

ed by the correlation coefficient squared is rather low in each case. Since in this population the males are larger and poorer feathered than the females (r = -0.28), it is to be expected that the coefficient of correlation would be higher when the sexes are considered separately.

DISCUSSION

A three-year experiment consisting of mating together individuals showing poor feathering at the broiler age reduced the mean degree of feathering to 1.5 in Rhode Island Reds. The majority of the males from this line fall in class 1, and the females in class 2. Mere selection of rapid feathering individuals at the broiler age has been less effective, the mean degree of feathering for the progeny of this pen being 2.0.

Crossbreeding in the case of the White Rock and Buff Orpington strains considered here, has increased the eight-week weight to that of the more rapid growing variety. Were there no effect of hybrid vigor, it would be expected that the eight-week weight of the crosses would be intermediate between that of their half-brothers and half-sisters in the standard varieties. In the reciprocal crosses feathering has shown very little, if any effect of hybrid vigor.

SUMMARY

When conditions of feeding and management are the same, the relative importance of some of the causes for variation in weight and feathering at eight weeks of age are as follows:

Cause of Variation	*Percent of Total Variance*	
	Weight	*Feathering*
Varieties	13	4
Pen (mostly sire)	19	11
Dam (when mated to one male)	27	16
Sex	23	20
Residual (non-heritable	18	49
Total	100	100

For both eight-week weight and feathering, differences between the means when chickens are grouped according to variety, sire, dam, and sex are highly significant.

Analysis of covariance between eight-week weight and feathering shows that these are correlated but the degree of determination is rather low.

Growth to eight weeks of age appears to be a separate entity and not necessarily related to adult weight.

Reciprocal crosses between a rapid-growing early-feathering variety and a slow-growing late-feathering one, have an eight-week equal to that of the rapid growing parental stock but are more or less intermediate in number of individuals well feathered at eight weeks of age.

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Loss of Weight of Hen's Eggs During Incubation Under Different Conditions of Humidity and Temperature

EMMA M. PRINGLE AND H. G. BAROTT
Bureau of Animal Industry, United States Department of Agriculture, Washington, D.C.

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THE progressive loss of weight of eggs during incubation has been known since the eighteenth century when Reaumer (1750) made his noteworthy studies on incubating eggs. Since that time many investigators have studied this phenomenon.

The many studies have proved that the chief source of weight loss is due to evaporation of water which depends on the relative vapor pressure inside and outside of the shell of the incubating egg. As the conditions inside the shell are practically constant, the loss of weight depends upon the vapor pressure in the environment of the egg which is controlled principally by temperature and humidity.

In early work no attempt was made to determine the humidity of the air surrounding the eggs, consequently the loss of weight reported varied greatly, ranging from 9 to 20 percent for 20 days.

Later investigators attempted to measure the humidity in the incubator while ascertaining the loss of weight. The eggs were incubated in ordinary cabinet-type incubators in most instances and the wide discrepancy in results indicates that measurements of humidity in the chamber were subject to considerable error. The humidity was determined in most cases by means of wet and dry bulb thermometers. It is impossible to measure humidity correctly by such means in the air space of the incubators used because of insufficient airflow past the wet bulb thermometer. Gravimetric determinations which were made in other cases are also subject to error due to such factors as condensation of moisture before absorption in the reagent, error in measurement of gas volume, measurement of temperature and pressure of gas at the time of measurement of volume.

Lamson and Kirkpatrick (1918) performed extensive studies on incubating eggs, using a specially modified incubator with accessory apparatus for controlling humidity, rate of ventilation, temperature, and other factors. With this apparatus they studied the loss of weight of approximately 9,600 eggs, over a wide range of humidity. They found that the loss of weight for 20 days ranged from 5.5 percent at 75 percent relative humidity, to 17.5 percent at 17.5 percent humidity.

Murray (1925) reported a research in which the loss of weight of eggs was obtained daily. He incubated the eggs in a water-jacketed copper thermostat provided with a constant inlet of warm fresh air. The humidity was determined by means of wet and dry bulb thermometers. For different conditions of humidity ranging from 16 to 93 percent, he observed a daily loss which varied linearly from 0.640 to 0.044 percent of the initial weight.

EXPERIMENTAL PROCEDURE

The investigation here reported was conducted at the National Agricultural Research Center at Beltsville, Maryland, to

determine the relationship between loss of weight of eggs during incubation and environmental conditions relative to humidity and temperature. It extended over a period of four years during which time 22 experiments were performed with observations on more than 2,200 eggs.

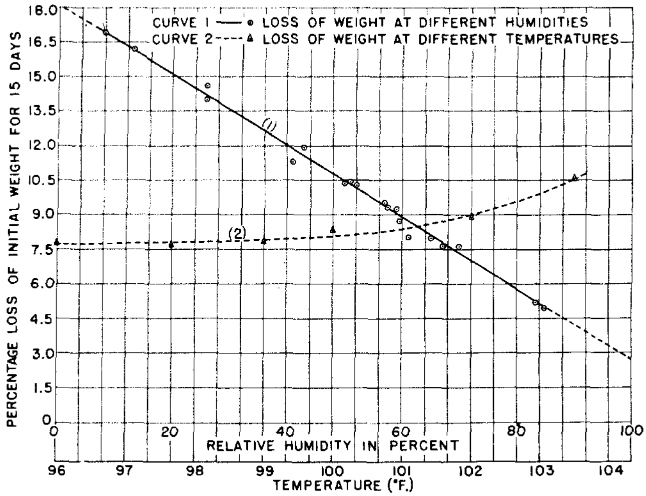

Fig. 1. Loss of weight of incubating eggs at different conditions of humidity and 102°F., and at different conditions of temperature and 60 percent relative humidity.

The eggs were obtained from selected flocks of White Leghorn hens which were trapnested. They were collected every three or four hours at which time each egg was plainly marked with the date and number of the hen that laid it. No eggs were incubated that were more than one week old.

The eggs were incubated in the two respiration calorimeters in the laboratory for physical measurements under accurately controlled conditions so that variability due to incubation conditions was minimal. The apparatus and methods have been described by Barott.[1] The temperature was kept constant throughout the incubation period so that the mean variation was less than 0.1°F. The humidity was determined from readings of a Lambrecht hair hygrometer checked every eight hours by a gravimetric determination. The mean variation in humidity was less than 0.1 percent. The oxygen content of the air was kept at 21 percent and the carbon dioxide content at less than 1 percent. The rate of airflow past the eggs was 12 cm. per minute which was sufficient to remove the moisture as fast as it was eliminated from the eggs.

[1] Barott, H. G. Effect of temperature, humidity, and other physical factors on the hatchability and energy metabolism of the chick embryo during incubation. (In course of preparation.)

The eggs were weighed at the start of incubation, on the seventh day, and on the fifteenth day. Each egg was weighed independently with an accuracy of 10 milligrams or with an accuracy of approximately 1 part in 5,000. The loss of weight was computed for each interval independently so that any change in rate of loss during the incubation period could be determined.

The investigations covered a range of humidity from 8 percent, which means exceedingly dry air, to 85 percent, which is very moist, at a temperature of 102° F. It also covered a range of temperature from 96° to 103.5° F. at a humidity of 60 percent.

Figure 1, curve 1, shows the relation of humidity to loss of weight during the first 15 days of incubation. The loss of weight decreased in direct proportion to the increase in the relative humidity and a change of 1 percent in humidity caused a change in loss of weight for 15 days of 0.15 percent or a mean loss of 0.01 percent per day for one percent change in humidity.

The results from the study to determine the effect of temperature on loss of weight are shown in Figure 1, curve 2. There was very little increase in loss of weight from 96° to 99° F., but from 99° to 103.5° F., the increase became progressively greater with each degree rise in temperature until at 103.5° the loss of weight was approximately one-third greater than at 99° F.

No difference in loss of weight of fertile and infertile eggs was noted during the first week of incubation. Inasmuch as all infertile eggs were removed at the first candling, no data were obtained beyond that time.

Murray (1925) found that the daily loss of weight of fertile eggs becomes greater as incubation proceeds. He says, ". . . changes taking place in fertile eggs result in a modification of the factors conditioning evaporation . . . the most important of which . . . is the amount of heat produced by the embryo." He states, ". . . a significant change (in loss of weight) does not become apparent until the last three or four days of incubation."

However, in this investigation it was noted that there was a significantly greater loss of weight in the period from the first to the second candling than in the period from the start of incubation to the first candling. The growing embryo is drawing upon the calcium in the shell and this may cause an increased porosity and consequently an increased evaporation. Table 1 shows the magnitude of the increase for these two periods.

TABLE 1.—*Increase in daily loss of weight of eggs incubated at 102° F. under different conditions of humidity*

Relative humidity	Mean daily loss of weight		Increase in daily loss of weight second period minus first period
	First period*	Second period†	
percent	percent	percent	percent
8	1.09	1.17	0.08
13	1.04	1.11	0.07
26	0.92	0.95	0.03
40	0.78	0.81	0.03
50	0.68	0.71	0.03
60	0.58	0.60	0.02
63	0.50	0.52	0.02
85	0.33	0.34	0.01

* First period—start to first candling.

† Second period—first candling to second candling.

The table shows that the increase in daily loss of weight is greatest at the lowest humidity, diminishing as the humidity increases.

The percentage loss of weight per day of 497 eggs was correlated with the weight of the eggs. The results of this correlation, based on the least squares method, are given in Table 2.

The negative coefficient of correlation obtained, —0.0963, indicates that the relationship between egg weight and percentage loss of weight is inverse. The coefficient of regression indicates a declining percentage

TABLE 2.—*Relation of egg weight to percentage loss of weight of egg per day. (497 eggs)**

Mean weight of eggs (grams)	54.12 ±0.1373
Standard deviation	4.5355±0.0971
Coefficient of variability	8.3805±0.1793
Mean loss of weight of eggs per day (percent)	0.5940±0.0023
Standard deviation	0.0762±0.0016
Coefficient of variability	12.8215±0.2743
Coefficient of correlation (r)	−0.0963±0.02997
Coefficient of regression (b)	−0.00162

* Relative humidity 60 percent; temperature 102°F.

loss of approximately 0.0016 for every gram increase in weight of egg. The greater loss of weight for small eggs may be due to the fact that there is a greater surface area per unit weight.

CONCLUSIONS

Loss of weight of fertile eggs during incubation depends principally upon the relative humidity of the environment. The loss decreases in direct prorportion to the increase in the humidity. An increase of 1 percent in the humidity causes the loss of weight to decrease 0.01 percent per day and vice versa.

From 96° to 99°F., temperature has but a slight effect on the loss of weight. However, from 99° to 103.5°F. a pronounced increase in loss of weight is noted. The loss of weight becomes progressively greater with each degree rise of temperature until at 103.5°F. the loss is one-third greater than at 99°.

Loss of weight increases as incubation proceeds.

The relationship between egg weight and loss of weight is inverse, the regression coefficient indicating a declining percentage loss of approximately 0.0016 for every gram increase in weight of egg.

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Augmentation of Nitrogen to the Egg White After the Formation of the Shell Membranes in the Fowl*

H. M. Scott, J. S. Hughes and D. C. Warren
Kansas Agricultural Experiment Station, Manhattan, Kansas

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FOLLOWING its discharge from the ovary, the yolk of the hen's egg is engulfed by the infundibulum of the oviduct. The ovum then enters the magnum where it is surrounded by a viscous layer of egg white. On leaving the magnum (albumen secreting region), the egg passes into the isthmian region, where it is enclosed by a very loose fitting membrane. The egg then enters the uterus where first the remainder of egg white (approximately 51 percent) is added, and then the entire egg is enclosed in a calcareous shell. This very briefly outlines the process of egg formation in the fowl for the period that the egg remains in the egg duct.

Pearl and Curtis (1912) concluded from their studies on the physiology of the oviduct that the secretion of egg white proteins is not restricted to any one division of the oviducal tube. They stated that only a part of the nitrogen could be accounted for as the egg passed from the magnum and that the remainder of the nitrogenous substance is taken into the egg through the shell membranes while the egg is in the isthmian and shell gland regions. The material thus passing through the shell membranes was referred to as a dilute albumen which constitutes the outer thin white of the fully formed laid egg.

This physiological problem has received little attention since that time. Chomkovic's (1927) work on the formation of egg white and the shell of duck eggs is probably the most comprehensive work undertaken on the physiology of egg formation since that of Pearl and Curtis. Although Chomkovic states that a little protein penetrates into the interior of the egg during its sojourn in the uterus, he offers no direct proof that this phenomenon actually takes place. The recent work of McNally (1934) tends to support the theory postulated by Pearl and Curtis. McNally states that approximately 60 percent of the absolute weight of the egg white and 90 percent of the nitrogen is accounted for at the time the shell membranes are formed in the isthmus. From an analysis of laid eggs and oviducal eggs, he concluded that the substance added by osmosis to the interior of the egg was a dilute solution of ovoglobulin with perhaps the salts necessary to keep this protein dispersed. He found no appreciable difference in the ovomucin, ovoalbumin, ovomucoid, and non-protein nitrogen content of mature (laid) eggs and immature (oviducal eggs).

It seems unlikely that the increased nitrogen content of the egg white of the laid egg over that of the immature egg coming from the same hen can be explained on the basis of the passage of ovoglobulins through the shell membranes. Hughes and Scott (1936) have shown that the greatest percentage of ovoglobulin for both the im-

* Contribution No. 97 from the Department of Poultry Husbandry and No. 205 from the Department of Chemistry.

mature and laid egg is found in the inner thin layer of white which surrounds the yolk mass. If the ovoglobulins pass through the membranes of the uterine egg, it is to be expected that the greatest concentration would be in the outer thin white. It was suggested that the increase in the amount of ovoglobulin of the laid egg over that of the uterine egg was due to a change in the solubility of the egg white proteins. We have found no difference in the ratio of non-protein to protein nitrogen in isthmian and freshly laid eggs from the same hen. Of the total nitrogen in the white of laid eggs the mean non-protein nitrogen value was found to be 4.26 percent and for the isthmian egg 4.24 percent. The relative amount of non-protein nitrogen should be reduced if ovoglobulin alone were added in the uterus.

Hansen (1934) asserts that the addition of proteins through the shell membranes "seems rather improbable since it must be assumed at the outset that the shell membrane is impermeable to albumen solutions." This investigator is of the opinion that a solution of salts penetrates the shell membrane. He was able to observe that a membranous egg having a viscous white, when placed in a physiological salt solution, quickly attained its normal plump size. Hansen did not, however, offer critical evidence that it was impossible for proteins to permeate the shell membranes nor prove that egg white proteins were not elaborated in the uterine region of the oviduct.

In support of the theory "that there is a definite addition of albumen to the egg in the isthmus and shell gland, and that the thin albumen layer does not represent solely a dilution of the dense layer" Pearl and Curtis offer the following pertinent facts:

1. The accumulation of egg white is continuous from the time the egg enters the magnum until it receives its full charge of egg white in the uterus.

2. Histologically the same kind of glands are found in the magnum, isthmus, and uterus.

3. The small amount of fluid that is free in the lumen of the uterus and which surrounds the membranous egg contains 0.22 percent nitrogen.

4. The absolute amount of nitrogen found in the laid eggs cannot be fully accounted for in eggs removed from the isthmus and uterus of the same hen.

Physiological studies made at the Kansas station would not indicate that egg white is added to the egg while in the isthmian region. This is in agreement with the work reported by Asmundson and Jervis (1933). They found that resection of the isthmian division of the oviduct exerted no influence on the amount or proportion of egg white secreted.

The fact that the same kind of glands are found in the isthmus, uterus, and the magnum does not preclude the possibility that they are physiologically different, although histologically similar.

We have, on a few occasions, collected small quantities of the fluid that surrounds the membranous egg shortly after the egg passed into the uterus. Chemical analyses show that this fluid contains protein and non-protein nitrogen. Since it is known that the matrix of the shell contains protein, there can be little doubt that some of the glands in this division of the egg duct have the function of secreting protein. It is entirely possible, however, that some of the nitrogen found in the lumen of the uterus has been secreted elsewhere in the oviduct for if the lumen of the magnum and isthmus is opened immediately following the passage of the ovum through these regions, a thin film of gelatinous material, egg white in nature, will be found adhering to the mucosa.

This is in accord with the histological findings of Richardson (1935). He has shown that the glands of the magnum con-

tinue to form egg white after the ovum has passed caudalward and stated that "there is no evidence against the contention that the residual secretion may drain down the oviducal walls to reach the egg below."

To test the validity of the theory that nitrogen is added to the egg in the uterus after it receives the egg membranes, Pearl and Curtis determined the amount of nitrogen in the egg white of oviducal eggs, extracted from various levels of the egg duct. The absolute nitrogen of the immature egg intercepted in the oviduct was expressed as a percentage of the absolute nitrogen found in the white of a laid egg from the same female.

STOCK AND PROCEDURE

The hens used in these experiments were White Leghorns in their first year of productivity. In general the technique employed by Pearl and Curtis was used.

In separating the egg parts for analyses, the bulk of the white for each egg was poured directly into a kjeldahl flask and the remaining white carefully separated from the yolk by rolling on filter paper. The filter paper used to absorb the white from the surface of the yolk, was added to the flask. The white adhering to the membranes of both the intercepted and laid eggs was washed into digestion flasks with distilled water or with 1 percent salt solution.

It is exceedingly difficult to make a complete separation of the yolk and white of the isthmian egg since the white at this stage of formation is very gelatinous. Moreover, the inner thin layer of white found in the laid egg usually is not present in the isthmian egg, and if present at all, only in minute quantities. In the fully formed egg, the inner thin white facilitates the separation of the yolk from the white. In a few cases, the yolks of the intercepted and laid eggs were washed in 1 percent saline after having been rolled on filter paper. Separate nitrogen determinations were run on these washings and although more nitrogen could be removed by washing the yolk of the isthmian egg than by washing the laid egg, the maximum amount of nitrogen that could be recovered thus was only 1.5 milligrams. This amount was too small to influence the results significantly. Since frequent handling of the yolk increased the danger of rupturing the vitelline membrane, and the weight of the yolk was desired, the washing operations were discontinued.

Since the relative amount of nitrogen in the white of the immature egg is greater than that of the laid egg, any loss that might have occurred by transferring the material to the flasks would probably be greater for the intercepted egg.

RESULTS

In Table 1 data are presented to test whether nitrogen is added to the egg white after the ovum had passed caudalward from the isthmus. All the intercepted eggs that appear in these data were removed from the isthmus. This was made possible by noting the time of the previous oviposition and then exploring the abdominal cavity and detecting by palpation the forming egg. This procedure has been described by Warren and Scott (1935) and made possible the recovery of the ovum from the isthmian region. In each instance the intercepted egg was second in clutch position while the control laid egg upon which the calculations were made was first in clutch position. This procedure eliminated all birds with single egg clutches but made it possible to use any females with clutches of two or more eggs.

Pearl and Curtis drew their conclusions from the analyses of eggs of 13 hens. Of this total, one of the intercepted eggs was in the magnum, two had not entirely cleared the magnum, three were in the isthmus, and the remaining seven were recovered from

the uterus. It is obvious that any egg which had failed to pass beyond the junction of the magnum and isthmus had not acquired its full quota of firm white and that of the total, only three eggs were recovered from the division of the egg duct that would offer critical evidence for the theory that nitrogen is added to the egg in the uterus after leaving the isthmian region.

larger than the control egg. This deduction is made from the fact that the yolks of the intercepted eggs were larger than the corresponding yolks of the laid eggs. Studies conducted in this laboratory indicate that intra-hen variations in egg size are due primarily to differences in yolk size. From the foregoing data it is apparent that either nitrogen is added to the white of the egg while it is in the uterus and thus must pass through the shell membranes or the method of approach to the problem has not taken into consideration certain fundamental factors.

TABLE 1.—*Egg white nitrogen of isthmian eggs (second in clutch position) expressed as a percentage of the nitrogen in the white of mature eggs laid by the same hen (first in clutch position)*

Hen No.	Weight in Grams						Percentage nitrogen accounted for
	Isthmian egg			Laid egg			
	Yolk	White	Nitrogen in white	Yolk	White	Nitrogen in white	
8	16.230	15.365	0.519	16.915	31.657	0.535	97.0
9	13.965	19.250	0.618	14.430	33.700	0.618	100.0
12	16.100	21.265	0.618	17.380	39.075	0.659	93.7
19	13.295	16.475	0.577	14.740	33.115	0.642	89.7
1174	14.730	13.840	0.457	14.572	28.555	0.461	99.1
1142	15.200	17.185	0.545	15.230	32.510	0.575	94.8
1133	13.935	13.990	0.425	14.225	30.233	0.452	94.0
9310	14.350	15.365	0.521	14.830	31.079	0.552	94.4
1240	13.350	—	0.538	13.205	32.060	0.535	100.6
1141	16.435	17.260	0.622	15.965	36.935	0.601	103.5
1222	14.535	18.990	0.570	15.455	35.090	0.582	97.9
857	16.270	14.910	0.342	17.192	30.452	0.359	95.3
867	15.979	15.769	0.271	15.495	31.765	0.306	88.6
Mean	14.952	16.639	0.509	15.356	32.847*	0.529	96.2

* Hen 1240 omitted in calculating this mean.

It is shown in Table 1 that the white of the isthmian egg contained on an average 96 percent of the nitrogen of the control laid egg. This quantity of nitrogen is accounted for even though the ovum had acquired only 50.7 percent of its egg white when intercepted. The mean intercepted egg contained 16.6 grams of egg white as compared with 32.8 grams for the laid egg. The nitrogen thus accounted for is approximately 6 percent higher than that reported by Pearl and Curtis and by McNally. In three instances 100 percent or more of the nitrogen was accounted for, and in two of these instances it was anticipated that the intercepted egg, had it been permitted to complete its development, would have been larger than the control egg.

THE PROBABLE EFFECT OF CLUTCH POSITION UPON THE RESULTS OBTAINED

Atwood (1929) and others have demonstrated that the position of the egg in the clutch exerts an influence on egg size. Table 2 of this communication also shows the relationship that exists between egg size and clutch position. Moreover the data would indicate that each component part of the egg is usually affected. In general, where

large numbers of eggs are weighed, the mean of each consecutively produced egg is smaller than its predecessor. However, when the record of an individual hen is examined, considerable variability is observed in the relative size of eggs in the various clutch positions. In the data of Table 1 each control egg was first while each intercepted egg was second in clutch position, and the latter because of its position should on an average, be smaller than the control egg.

From the appendix of Pearl and Curtis' paper, it is clearly evident that in each case the intercepted egg analyzed was at least the second egg of the clutch and in some instances the eighth (hen 8005). Where we have used the single laid egg upon which to base our calculations, Pearl and Curtis have used the mean of several laid eggs from the same hen and have in this way somewhat compensated for differences in egg size. Nevertheless, of the eight hens in which the oviducal egg had cleared the magnum and on which they submit data relating to yolk weight, the mean yolk weight of the laid eggs for each hen was greater with one exception (hen 8033) than the weight of the intercepted egg. The interpretation placed by Pearl and Curtis upon their data was "what the figures from the analyses of 13 oviduct eggs show is that with four exceptions, the oviduct egg has absolutely less nitrogen in its albumen than the normal laid egg of the same hen." It is interesting to note that in each of the four cases where the immature egg contained more nitrogen than the laid egg, the former was recovered from the uterus. It is true that in these four cases the intercepted eggs had been in the uterus for some period of time and had accumulated most if not all of their egg white. They predicate that these four exceptions "are undoutbedly to be explained as the result of fluctuation of the absolute size of the egg." They take cognizance of the fact "that if the oviduct egg happens to be an exceptionally large one, relative to the other eggs of the same bird, it may have a slightly greater absolute amount of nitrogen though not yet laid, than another relatively or absolutely smaller laid egg taken for a control." It should be pointed out, however, that the reverse may also be true.

Eight females were selected to study the influence of clutch position on the nitrogen content of consecutively laid eggs. The data on the weight of the egg parts and on the analyses of the white are given for 49 eggs in Tables 2 and 3. The clutch size of these females varied from two to five eggs. A total of 15 clutches where the first and second eggs are represented, appear in these data. In each of the 15 instances the white of the second egg of the clutch contained less absolute nitrogen than the white of the first egg. The average second egg of the clutch contained 90.08 percent of the nitrogen of the first egg (Table 3). Thus, under these experimental conditions, although the second egg of the clutch was permitted to reach completion, there still remained 10 percent of the nitrogen to be accounted for. This amount is identical with the quantity of nitrogen that McNally concluded must be added following the formation of the shell membranes. Had the second egg been intercepted in the isthmus, the data could be interpreted to indicate that the 10 percent nitrogen unaccounted for, would have been added as the egg moved caudalward from the isthmus. In only one of the 15 instances cited was the second egg of the clutch larger than the first egg (hen 847, Table 2). It is obvious, therefore, that where the nitrogen of the second egg is to be expressed as a percentage of the nitrogen found in the white of the first egg, that clutch position insofar as it affects egg size, must be considered in interpreting the results. This would hold true whether the second egg is

TABLE 2.—*The percentage nitrogen in the whites of consecutive eggs of a hen*

Hen No.	Clutch No.	Position of egg in clutch	Weight of egg and parts (gm)				Grams of nitrogen in white	Percentage of nitrogen in white
			Total	Yolk	White	Shell		
		1	57.600	16.337	36.02	5.244	0.557	1.55
856	1	2	56.320	15.965	35.04	5.316	0.492	1.40
		3	50.190	14.327	30.86	4.900	0.462	1.50
	2	1	53.705	14.440	34.45	4.817	0.535	1.55
		1	56.605	18.822	32.61	5.175	0.529	1.62
	1	2	56.190	18.694	32.44	5.055	0.488	1.50
843		3	52.797*					
	2	1	56.017	17.789	33.25	4.975	0.542	1.63
		2	53.825	17.968	31.30	4.560	0.471	1.50
	1	1	56.650	17.139	33.83	5.683	0.488	1.44
		2	53.338	15.803	31.97	5.563	0.475	1.49
	2	1	55.275	15.633	34.27	5.368	0.524	1.53
958		2	52.039*					
		1	55.277	16.523	33.19	5.562	0.518	1.56
	3	2	54.482	16.867	33.02	4.597	0.482	1.46
		3	53.154	16.279	31.56	5.312	0.447	1.42
		1	51.560	16.480	30.41	4.674	0.445	1.46
		2	48.236	16.225	27.46	4.550	0.392	1.43
	1	3	47.794	16.215	27.01	4.570	0.382	1.41
		4	47.342	15.449	27.15	4.741	0.389	1.43
845		1	52.102	16.142	31.01	4.951	0.474	1.53
		2	49.315	15.499	29.04	4.772	0.420	1.45
	2	3	46.282	15.026	26.76	4.501	0.396	1.48
		4	46.202	14.514	27.10	4.593	0.397	1.46
	3	1	53.182	15.658	32.61	4.912	0.467	1.43
		2	46.050	14.449	27.39	4.210	0.387	1.41
		1	63.047	17.405	39.71	5.935	0.682	1.81
	1	2	60.482	16.702	37.87	5.912	0.536	1.42
		3	57.362	15.739	35.82	5.800	0.532	1.49
852		1	60.827	16.625	38.37	5.832	0.700	1.82
	2	2	59.227	16.337	36.70	6.192	0.621	1.69
		3	55.667	15.429	34.72	5.514	0.597	1.72
	3	1	60.165	16.218	38.45	5.501	0.719	1.87
		2	57.261*					
		1	49.719	14.268	31.25	4.199	0.491	1.57
	1	2	49.180	14.723	30.13	4.332	0.445	1.48
960		3	47.865	14.190	29.24	4.431	0.422	1.44
	2	1	49.363	14.704	31.07	3.586	0.463	1.49
		2	48.910	15.243	29.93	3.741	0.451	1.51
	3	1	51.457	14.642	33.21	3.610	0.512	1.54
	1	1	52.927	16.357	31.15	5.420	0.487	1.56
		2	51.433	16.805	29.49	5.141	0.446	1.51
		1	53.983	17.364	31.97	4.645	0.496	1.55
9596	2	2	52.559	17.492	30.07	5.000	0.458	1.52
		3	50.738	16.180	29.80	4.762	0.448	1.50
		4	51.041	17.035	29.63	4.331	0.453	1.53
	3	1	53.014	16.657	32.46	3.890	0.478	1.47
		1	57.789	15.805	37.21	4.777	0.670	1.80
		2	58.590	15.899	37.39	5.302	0.643	1.72
847	1	3	53.974	15.299	34.04	4.632	0.610	1.79
		4	54.662	15.590	34.25	4.820	0.563	1.64
		5	52.292	14.514	33.15	4.630	0.544	1.64

* Destroyed in separating egg parts.

fully formed and laid or only partially formed and intercepted in the isthmus.

The data of Table 2 would indicate that a factor other than egg size is in part responsible for the decrease in the nitrogen content of the white of the egg second in clutch position. Reference has been made to hen 847 wherein the egg second in clutch position was larger than the first. Each component part of the second egg was larger than the corresponding part of the first egg. Although the amount of white of the second egg was greater than the white of the first egg, the former contained less absolute nitrogen. This would suggest that the concentration of nitrogen in the whites of consecutively laid eggs may be influenced by clutch position. In the last column of Table 2 the percentages of nitrogen in the whites of each egg are given. In 13 of the 15 clutches where the first and second eggs are represented, the percentage of nitrogen in the white of the second egg was less than the percentage of nitrogen in the first egg of the clutch. The two exceptions are found in the second clutch of hen 960 and the first clutch of hen 958.

The mean percentage nitrogen in the white of the first egg for the 15 clutches cited was found to be 1.58 and for the second egg 1.50. The difference, 0.08 percent suggests that the white of the second egg contains 5.06 percent ($\frac{.08}{1.58}$ x 100) less nitrogen per unit weight of white than does the white of the first egg. While the data are meager, there is some evidence from the appendix of Pearl and Curtis' paper that supports this viewpoint. The eggs laid March 16 and 17 by hen 8010 and May 23 and 24 by hen 8018 show this relationship between the two eggs in the data of these authors.

TABLE 3.—*The effect of clutch position on the nitrogen content of the white of consecutively laid eggs*

Hen No.	Clutch No.	Grams of nitrogen in white		$\frac{\text{Nitrogen of second egg}}{\text{Nitrogen of first egg}} \times 100$
		First egg	Second egg	
856	1	0.557	0.492	88.3
843	1 2	0.529 0.542	0.488 0.471	92.2 86.9
958	1 3	0.488 0.518	0.475 0.482	97.3 93.1
845	1 2 3	0.445 0.474 0.467	0.392 0.420 0.387	88.1 88.6 82.8
852	1 2	0.682 0.700	0.536 0.621	78.6 88.7
960	1 2	0.491 0.463	0.445 0.451	90.6 97.4
9596	1 2	0.487 0.496	0.446 0.458	91.6 92.3
847	1	0.670	0.643	96.0
Total	15 pairs		Mean......................	90.2

DISCUSSION

The data presented show that the average absolute amount of nitrogen in the white of the isthmian egg is less than the absolute

amount of nitrogen in the laid egg when the former egg is second in clutch position and the latter the first egg of the clutch. It seems unnecessary to explain this difference on the basis that additional nitrogen will be added to the white in the uterine region. The difference can in all probability be attributed to the effect of clutch position. The second egg of the clutch is smaller than the initial egg of the clutch. Moreover, the relative amount of nitrogen in the white of the first egg is usually greater than that of the second. This relationship seems to hold true between the first and all succeeding eggs of the clutch. It is not fully understood why the second egg of the clutch is smaller than the first egg. It has been suggested by some workers that the egg forming organs become exhausted when eggs are laid consecutively and that this is responsible for the decrease in egg size. Scott and Warren (1936) have demonstrated that when the clutch is terminated, the oviduct undergoes a rest period of approximately 15.5 hours. Following this period of rest, the initial egg of the succeeding clutch is not only larger but contains a greater concentration of nitrogen in the white than subsequent eggs of that clutch. These observations would tend to support the theory of fatigue. The rest period referred to has been used in the sense that no egg is present in the oviduct. The 15.5 hour period represents the time elapsing between the oviposition of the last egg of a clutch and the ovulation of the first egg of the succeeding clutch. Richardson (1935) has recently made a comprehensive histological study of the fowl's oviduct while in various stages of activity. He concluded that the albumen region contains glands which are always in the process of secreting and therefore, the 15.5 hours may not be a true period of rest. However, it is not clear from the text of his paper whether this condition occurs only when consecutive eggs are moving through the oviduct or to the period that follows a break in the clutch as well.

If an experiment were outlined so as to intercept the first egg of a clutch in the isthmus and to use as a control the last egg of the preceding clutch, it probably could be demonstrated that the isthmian egg contains more absolute nitrogen than the control laid egg.

Aside from the evidence that has been presented thus far, certain laws of physical chemistry indicate that protein solutions would not pass through the shell membranes in the direction that Pearl and Curtis and McNally say they do. In discussing this point, Pearl and Curtis state, "That the egg does not take water from the blood by osmosis; and in this way dilute the dense albumen to form the thin is further evidenced by the fact, shown by Atkins that in the domestic fowl the osmotic pressure of the blood is very considerably higher (nearly two atmospheres) than the osmotic pressure of the egg. In any osmotic exchange under these conditions, water would tend to pass from the egg to the blood and not in the other direction." However, it should be pointed out that the blood does not come in contact with the shell membrane. The condition described by Pearl and Curtis does not exist in the uterus and the osmotic pressure of the blood in no way influences the movement of fluids through the shell membranes. The uterine secretion, which is separated from the egg white by the shell membranes, determines the passage of water into the egg. Since these membranes are readily permeable to electrolytes but almost impermeable to colloids, the difference in colloidal osmotic pressure and not the total osmotic pressure would determine the direction of the passage of water. The white of an isthmian egg contains approximately 20 percent protein. Since Beadle, Hughes, and Scott (1936) have found that the uterine secretion contains

about 0.4 percent protein, it is evident that water would pass from the uterine fluid of low colloidal osmotic pressure through the shell membranes into the white of much greater colloidal osmotic pressure. Since the *concentration of protein is many times* as great in the egg white as in the uterine secretion, energy would be required to force protein from the uterine secretion into the egg through the shell membranes. The membranes are of course, devoid of any means of developing this energy and it is, therefore, unlikely that any protein is added to the white of an egg in the uterus. In fact if these membranes are slightly permeable to egg white proteins, one would expect the proteins to pass from the egg white, through the shell membranes into the uterine secretion.

SUMMARY

1. The absolute amount of nitrogen was determined for immature eggs (isthmian eggs) and laid eggs from the same individual. In each case the laid egg was first in clutch position while the intercepted egg was the second egg of the same clutch. It was found that the intercepted egg contained 96 percent of the nitrogen of the laid egg and that the former had acquired by weight 51 percent of the white of the laid egg.

2. The whites of consecutively laid eggs from eight females were analyzed for total nitrogen. Laid eggs, second in clutch position, contained only 90 percent of the nitrogen of the first eggs of the clutch. This difference is due to the greater size of the first egg and a greater concentration of nitrogen in its white.

3. This difference in nitrogen content between consecutively laid eggs is as great as that reported by previous investigators between isthmian and laid eggs upon which they base the contention that protein is added to the egg through the shell membranes.

4. A consideration of the laws of physical chemistry leads to the conclusion that proteins *would not pass from the uterine secretion* of low protein concentration through the shell membranes into the egg white of higher protein concentration.

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Development of Fertility in Young Barred Plymouth Rock Males*

R. L. Hogue and E. E. Schnetzler
Department of Poultry Husbandry, Purdue University, Lafayette, Indiana

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IT HAS been observed on the Purdue Poultry Farm that a larger number of infertile hatching eggs are obtained during the late summer and early fall months when the mature males are molting. Upp and Thompson (1927) in their study of seasonal hatchability made similar observations and found that during the period from October 20 to November 3 the fertility was only 63.5 percent.

Jaap (1933) reports that during the late winter and early spring the primary reproductive organs of the Mallard drake normally increase in size and spermatogenic activity. He found that the testes decreased in size at the end of the laying season and that molt follows. Microscopic studies of the gonads of thyroid-fed drakes showed that the largest testes had a large number of mature sperms in the lumen of each tubule, while the smaller testes showed only mitotic division.

Since some poultrymen are now setting eggs during the late summer and early fall months, it is important economically to find some means of keeping the fertility at an optimum level during this period. The object of this study was to determine at what age Barred Plymouth Rock males give satisfactory fertility. It might be possible, if sexual maturity is reached at an early age, to use the young males that are being saved for the next season's breeders, and obtain better fertility than if old males are used.

EXPERIMENTAL

Fifty-three day-old Barred Plymouth Rock chicks were started November 7, 1935, in the laboratory on brooding tables described by Carrick (1925). These chicks were fed a standard starting ration and reared indoors to 20 weeks of age. At the end of each two-week period body weights were taken and two of the males killed and their comb and testes removed. The comb and one of the testes were weighed and preserved for a study of the comparative increase in size of these organs at the different ages. The other testis was prepared for histological studies to determine the age when spermatozoa could be found. A small portion of each organ, taken near the center of the short axis, was fixed in Bouin's fluid and embedded in paraffin. Each section was cut five microns and stained with iron hematoxylin described by Jordan (1930.)

To study the age when fertile eggs would first be obtained, individual matings of 16 Barred Plymouth Rock males, 14 weeks of age, were made. Eight of the males were placed in single exhibition coops with laying Barred Plymouth Rock pullets and eight with White Leghorn pullets in laying condition. Since the White Legborn pullets were nearer the body size of the males it was thought that copulation would occur at an earlier age. The males used in these matings were those showing the largest comb and wattle development out of a

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brood of 23. All eggs obtained from these matings were marked and incubated for seven days; afterwards the eggs were broken to determine fertility.

To determine the age at which satisfactory fertility would be obtained from pen matings, 14 Barred Plymouth Rock males, 19 weeks of age, were divided equally in seven pens of Barred Plymouth Rock pullets just coming into production. These cockerels were selected from 26 males which had been fed a standard ration and brooded in the laboratory. The size of the comb and wattles was used in making the selections. After the males had been in the pens for one week, all of the eggs were incubated for seven days and then broken to determine the fertility.

RESULTS

The microscopic studies of the sectioned testes show mitotic figures in the tubules of the males six weeks of age. It can be seen from Figures 1 and 2, males six and eight weeks of age, that mitosis was beginning in the peripheral cells and that spermatogenesis had not developed beyond the spermatogonium and the prophase stage of the primary spermatocyte. There was no apparent difference in the germ cell development in the two testes sectioned at eight weeks, although there was a difference of about 200 grams between the body weights of the two males from which the organs were taken.

Figures 3, 4, 5, and 6 are transections of the seminiferous tubules of males 10, 12, 14, and 16 weeks of age respectively. These figures show the increase in size of the tubules and the advance in the stages of mitosis with the age of the males. Figure 3, male 46, 10 weeks of age, shows that mitotic division is confined largely to the peripheral cells and apparently mitosis has not developed beyond the primary spermatocyte. The sections of the testes from the males killed at 12 and 14 weeks do not show outstanding differences in spermatogenesis. Many phases of mitotic division can be found, but no spermatids are apparent. From the size of the tubules and the germ cell development in the 16-week old male, Figure 6, it appears that spermatogenesis is almost complete. However, it was impossible to find mature sperms in the sections taken at approximately mid-point through the short axis.

TABLE 1.—*Weights of the body, testis, and comb at different ages*

Male No.	Age	Body wt.	Testis wt.	Comb wt.
	wks.	gms.	gms.	gms.
34	4	180	.02	.075
95	4	221	.01	.05
19	6	261	.03	.11
92	6	351	.08	.58
84	8	652	.07	.38
93	8	910	.06	.40
46	10	1052	.16	.70
68	10	823	.16	.40
31	12	1043	.17	1.15
88	12	1210	.12	.62
30	14	1755	.25	1.48
99	14	1340	.24	1.35
38	16	1542	.82	2.48
62	18	1925	3.56	3.27
87	18	1660	6.81	5.18
66	20	2120	9.44	3.75
89	20	2090	2.24	5.50

Figures 7 and 8 are transections of the seminiferous tubules of males 18 and 20 weeks of age. Each tubule contains a large number of spermatids and spermatozoa, and apparently there was considerable spermatogenic activity at this age. Smears made of the testicular fluid from these males, obtained by pressing the cut end of the testicle immediately after being removed, showed sperms that were free from the sertoli cells. A small number of these sperms were active.

Table 1 shows that the size of the testes and the comb increases with age. The rate of increase in size of these two organs is not in every instance the same. At 20 weeks in male 66 the testis was two and one-half times as heavy as the comb, while in

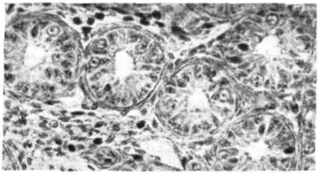
Fig. 1

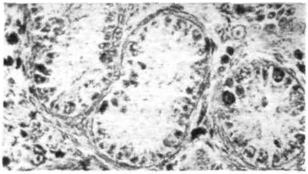
Fig. 2

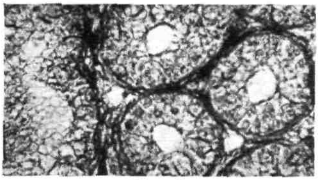
Fig. 3

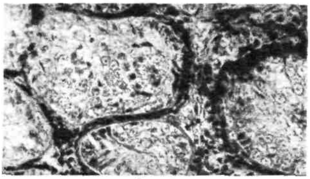
Fig. 4

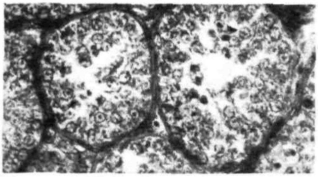
Fig. 5

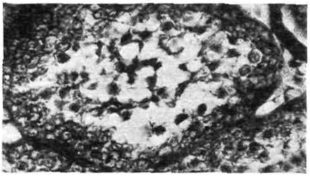
Fig. 6

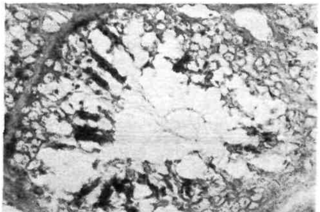
Fig. 7

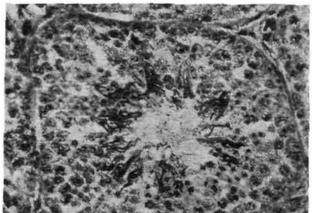
Fig. 8

(Photographs by E. J. Kohl)

(See opposite page for legends)

all of the other males the weights of the testis prior to 18 weeks were considerably less than that of the comb. The testes taken from the males killed after 16 weeks of age were considerably heavier in relation to the body and comb size than those taken from the males at earlier ages.

Individual differences in the growth of the testes and the combs of the chickens, of the same age and approximately the same weight, are also shown in Table 1. At 20 weeks of age, male 66 weighed 30 grams more than male 89, but the testes of male 66 were about four times heavier than those of male 89. The microscopic studies show the lumen of the tubules of male 66, which had the largest testis, to contain a larger number of mature sperms.

The results of the individual matings are given in Table 2. From the individual matings of the Barred Plymouth Rock males to the Barred Plymouth Rock females it was learned that some of the males could fertilize eggs at as early an age as 16 weeks, while others did not fertilize eggs until they were 32 weeks of age. In the matings of Barred Plymouth Rock males to White Leghorn females, two males fertilized eggs at 16 weeks of age. The age required for the other males varied up to 32 weeks, which was the maximum age in the pen matings of Barred Plymouth Rock males and females. Four of the 16 males started were removed because of poor health.

TABLE 2.—*Age of males when fertility was obtained from the individual matings*

Mated to B. P. Rocks		Mated to Leghorns	
Male No.	Age when fertility was obtained	Male No.	Age when fertility was obtained
	weeks		weeks
1	16	2	30
3	R*	4	26
5	32	6	16
7	R*	8	16
9	R*	10	26
11	27	12	18
14	18	13	32
16	R*	15	32

* Removed because of poor health.

Table 3 gives the results of the pen matings. The first fertile eggs were obtained from pen C, when the males were 21 weeks of age. Only one of the three eggs laid during the twenty-first week was fertile.

FIG. 1. Male 92, six weeks of age. The seminiferous tubules at this age are relatively small. A distinct row of spermatogonia can be seen next to the basement membrane of each tubule. ×340.

FIG. 2. Male 84, eight weeks of age. In this male the seminiferous tubules are larger, but spermatogenesis has not apparently developed much farther than in male 96, six weeks of age. A large number of the tubules appear to have more fully developed spermatogonia. ×340.

FIG. 3. Male 46, 10 weeks of age. In this section mitosis is not confined only to those cells around the outer edge of the tubule. Some of the cells next to the lumen show mitotic figures which indicate advancement of spermatogenesis. ×340.

FIG. 4. Male 31, 12 weeks of age. The seminiferous tubules of this male are larger than those of male 46, 10 weeks of age. Many of the cells contain mitotic figures and mitosis can be seen in the cells near the center of the tubule. ×340.

FIG. 5. Male 99, 14 weeks of age. With the exception of an increase in the number of cells showing mitotic figures, there was no apparent advancement of mitosis. ×340.

FIG. 6. Male 38, 16 weeks of age. There was apparently an increase in size of the tubules. This section shows advanced stages of spermatogenesis but no spermatids were found. ×340.

FIG. 7. Male 62, 18 weeks of age. This section shows considerable spermatogenic activity. Fully developed spermatozoa, darkly stained areas near the lumen, can be seen in the tubules. All phases of spermatogenesis were occurring at this age. ×290.

FIG. 8. Male 66, 20 weeks of age. There is no apparent change in spermatogenic activity from that shown in Figure 7, male 62, 18 weeks of age. All of the successive levels of spermatogenesis are clearly shown. ×290.

During the next week fertile eggs were obtained from pens D and H and by the time the males were 25 weeks of age fertile eggs were being obtained from all pens, except pen B. This pen did not produce fertile eggs until the males were 30 weeks of age. In pen G fertility was obtained from males when 24 weeks of age, but after the next week no fertile eggs were produced until the males were 30 weeks of age.

TABLE 3.—*Age of males when fertility was obtained from the pen matings*

Pen	Age in weeks												Age when fertility was obtained
	21		22		23		24		25		26		
	Eggs set	Per-cent fert.	Eggs set	Per-cent fert.	Eggs set	Per-cent fert.	Eggs set	Per-cent fert.	Eggs set	Per-cent fert.	Eggs set	Per-cent fert.	
													wks.
B	13	0	20	0	14	0	13	0	16	0	10	0	30
C	3	33	10	0	6	50	9	66	8	50	12	66	21
D	10	0	17	17	13	53	5	80	7	85	*		22
E	10	0	19	0	9	0	3	0	4	75	8	87	25
F	7	0	7	0	17	29	12	66	10	70	8	87	23
G	10	0	18	0	13	0	7	14	7	14	6	0	24
H	5	0	11	54	23	56	22	75	19	89	*		22

* Eggs were not saved after males were 25 weeks of age.

It can also be seen from Table 3 that a fairly satisfactory level of fertility was obtained from most of the males when they were 26 weeks of age. In four out of the seven pens the fertility ranged from 85 to 89 percent at this age.

DISCUSSION

The results from the histological studies of the testes and the individual and pen matings of this experiment have indicated that Barred Plymouth Rock males approach sexual maturity when 16 weeks of age. Only the largest and most mature males can be expected to produce fertile eggs at this age. If the stock were grown on range under more favorable conditions, sexual development might be more rapid than that found, in this experiment where the stock was reared indoors. It seems probable that satisfactory fertility may be obtained when cockerels are 26 weeks old, provided they have been fed adequate rations and given good management.

The poultryman who is having difficulty in securing fertile eggs during the late summer and early fall months, might find it profitable to use young males to replace the mature cocks, which are usually molting during that period. It does not seem advisable to put the males with the breeding flock before they are 26 weeks of age. Poultrymen should realize that there are wide individual differences in the rates of sexual development between males and the ratio of males to females should probably be lowered to allow for these differences.

There is some evidence from this study, which indicates a relationship between the development of the primary and secondary sexual characters of young Barred Plymouth Rock males. After 16 to 20 weeks of age the males with the largest combs and wattles, within a brood, are more likely to fertilize eggs than those having smaller combs and wattles. Possibly the size of the comb and wattles may be used as a practical criterion for selecting the males.

Since the Barred Plymouth Rock females to which the males were mated were ap-

proximately 36 weeks of age, it does not seem that males over 26 weeks of age would be handicapped in copulation because of insufficient size. Evidence for this belief is further supplemented from the matings with White Leghorn pullets which failed to give fertile eggs at an earlier date than the matings with Barred Plymouth Rock pullets.

No attempt has been made to study the viability of the germs, or the effects of early mating of the males upon their development. Additional studies of these two factors are needed.

SUMMARY

1. The results from the histological studies and the individual matings of this study indicate that Barred Plymouth Rock males may reach sexual maturity at approximately 16 weeks of age. There seems to be wide individual differences, depending upon the development of the males.

2. It appears that a satisfactory level of fertility may be secured from pen matings after the males are 26 weeks of age.

3. Poultrymen might find it advisable to use young early hatched males in the breeding pens during the late summer and early fall months to replace old males, which are likely to be molting and not producing satisfactory fertility.

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The Effects of Storage on the Vitamin D Value of Columbia River Salmon Fish Meal*

J. S. CARVER, VICTOR HEIMAN AND J. L. ST. JOHN
Divisions of Poultry Husbandry and Chemistry, Washington Agricultural Experiment Station, Pullman, Washington

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THE consumption of fish meals for poultry feeding has rapidly increased during the last few years. In the past, fish meals have been used principally for their protein values. No work on the ricket-preventing value of salmon fish meals has come to the attention of the authors of this paper, although work has been reported on the feeding of other fish meals with high or low fat content.

Dove (1934) in the study of Maine vacuum dried sardine meal found "that after eight months' storage, the sample containing the highest fat content produced results that still ranked first and tested the highest for vitamin D potency. Except for the third sample, the vitamin D value ranks in decreasing order with decreasing fat content." On the contrary, Allardyce, Henderson, and Asmundson (1933) stated that better growth was obtained from low fat content pilchard meals.

Carver, Brunstad, St. John, Frasier, and Athow (1933), in vitamin D studies with sardine oil, found that in several cases complete protection from rickets was obtained on rations in which 5 percent Alaska herring fish meal furnished the only source of vitamin D.

Many salmon and other fish meals contain considerable quantities of fat or fish oil. The amount of fat contained in fish meal depends on the variety of fish and the methods of extraction. In many poultry rations, supplementary oil extracted from various fish by-products is added and mixed in the feed as a source of vitamins A and D. If satisfactory feeding results could be obtained, it might be better to slightly increase the percent fat in the fish meals rather than to remove the oil from the fish meal and later add it to the poultry ration.

The purpose of the experiment reported here was to determine the vitamin D value of a high fat salmon fish meal and the effect of a year's storage of the salmon fish meal on its vitamin D value.

EXPERIMENTAL

The data reported here are based on two eight-week experiments with salmon fish meal. The first experiment started January, 1935, with six lots in duplicate, using Single Comb White Leghorn day-old pullets. The pullets were brooded in lots of 15 each for eight weeks in electrically heated battery brooders in the nutrition laboratories of the poultry department in an artificially lighted laboratory with curtained windows which prevented the admission of ultraviolet light. The Columbia River salmon

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TABLE 1.—*Composition of rations fed January, 1935*

Lot No.	1, 7	2, 8	3, 9	4, 10	5, 11	6, 12
Ingredient	percent	percent	percent	percent	percent	percent
Basal mash	74.5	73.6	75.1	74.7	75.9	78.4
Salmon fish meal[1]	—	—	0.7	1.4	2.9	5.7
Steamed bone meal	2.1	2.1	2.0	1.9	1.8	1.5
Salmon fish oil	—	0.5	—	—	—	—
Dried skimmilk	23.4	23.8	22.2	22.0	19.4	14.4
Total[2]	100.0	100.0	100.0	100.0	100.0	100.0

[1] The analysis of the salmon fish meal used in this experiment was: 17.4 percent fat; 51.0 percent protein on a moisture basis; 5.2 percent calcium; 3.1 percent phosphorus, and 9.5 percent moisture.
[2] All rations were standardized at a level of 17.0 percent protein, 1.0 percent calcium, and 0.8 percent phosphorus.

fish meal used was prepared under experimentally controlled conditions by the technological laboratory of the Bureau of Fisheries in Seattle, Washington.[1] The salmon meal was prepared from Columbia River salmon cannery waste by the usual wet process of reduction except that cooking and pressing were regulated to permit greater oil retention in the fresh residue. The meal was steam dried at a temperature of 212°F. The percent fat in the meal was 17.4; protein, 51.0 on a moisture basis; calcium, 5.2; phosphorus, 3.1; and the the moisture, 9.5.

[1] The meal was prepared under the supervision of R. W. Harrison, associate technologist-in-charge, Bureau of Fisheries, Technological Laboratory, Seattle, Washington.

The following basal ration of Carver, Brunstad, St. John, Frasier, and Athow (1933) was used in both experiments: 67.0 pounds ground yellow corn, 26.0 pounds wheat flour middlings, 6.5 pounds wheat bran, and 0.5 pounds salt. These all-mash rations were all standardized at a level of 17.0 percent protein, 1.0 percent calcium, and 0.8 percent phosphorus by the addition of varying amounts of dried skimmilk and steamed bone meal.

Rations used in the first year's experiment are described in Table 1. The positive control duplicate lots 2 and 8 were fed the basal ration with 0.5 percent of special Columbia River salmon oil prepared from total Chinook offal. Unpublished data from this experiment station has shown that this

TABLE 2.—*Composition of rations fed January, 1936*

Lot No.	13, 21	14, 22	15, 23	16, 24	17, 25	18, 26	19, 27	20, 28
Ingredient	percent	percent	percent	percent	percent	percent	percent	percent
Basal mash	74.5	73.6	74.7[1]	75.9[1]	78.4[1]	74.7[2]	75.9[2]	78.4[2]
Salmon fish meal[3]	—	—	1.4	2.9	5.7	1.4	2.9	5.7
Steamed bone meal	2.1	2.1	1.9	1.8	1.5	1.9	1.8	1.5
Salmon fish oil	—	0.5	—	—	—	—	—	—
Dried skimmilk	23.4	23.8	22.0	19.4	14.4	22.0	19.4	14.4
Total[4]	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1] This feed was mixed January, 1935, and stored until January, 1936.
[2] The basal ration used was mixed January, 1935, with the salmon fish meal stored separately and mixed with the ration January, 1936.
[3] The analysis of the salmon fish meal used in this experiment was: 17.4 percent fat; 51.0 percent protein on a moisture basis; 5.2 percent calcium; 3.1 percent phosphorus; and 9.5 percent moisture.
[4] All rations were standardized at a level of 17.0 percent protein, 1.0 percent calcium, and 0.8 percent phosphorus.

salmon oil would give excellent growth and supply sufficient amounts of vitamin D to protect from rickets when fed at this level.

The descriptions of the rations used in the second year's experiment are found in Table 2. There were eight duplicate lots. Lots 13 and 21, the negative control, and lots 14 and 22, the positive control, were mixed from the same ingredients used the previous year. The rations for lots 15 and 23, 16 and 24, and 17 and 25 were mixed in January, 1935, and stored until January, 1936, in a feed room protected from the direct sunshine with a temperature varying from 10°F. to 105°F. The rations fed to lots 18 and 26, 19 and 27, and 20 and 28 consisted of the basal mash mixed in January, 1935, with the salmon fish meal stored separately and mixed with the basal ration in January, 1936.

The day-old pullets in both experiments were individually weighed at the beginning of the experiment and each week thereafter. In both experiments, the vitamin D studies were continued for eight weeks. Records were kept of mortality and its causes, feed consumption, and feed analysis. In both experiments, the degree of calcification was determined by splitting longitudinally at right angles to the median ridge, the proximal ends of the left tibia of each chick. All tibia bones that were properly calcified were classified as normal, and all tibia bones that were not properly calcified were identified as having rickets. In the second year's experiment, the percent bone ash of

TABLE 3.—*Summary of 1935 results at eight weeks*

Lot No.	Vitamin D carrier fed	Source of vitamin D	Av. feed consumed per chick	Grams of feed per gram of gain	Average chick weight	Av. wt. of chicks in duplicate lots	Av. wt. of positive control	Degree of calcification	Mortality
	percent		grams		grams	grams	percent		
1 7	0.0	None	953.7 839.8	3.9 3.9	276.8 247.0	261.9	43.9	R[1] R	0 2
2 8	0.5	Salmon fish oil	1574.2 1694.0	2.8 3.0	592.9 599.8	596.3	100.0	N[2] N	0 0
3 9	0.7	Salmon fish meal	1162.8 974.6	3.6 3.7	356.7 297.7	327.2	54.9	R R	0 0
4 10	1.4	Salmon fish meal	1349.3 1322.7	3.5 3.4	419.1 420.0	419.5	70.3	R R	0 0
5 11	2.9	Salmon fish meal	1606.3 1608.7	2.9 3.2	579.5 538.7	559.1	93.8	N N	0 0
6 12	5.7	Salmon fish meal	1686.8 1698.7	2.9 3.2	611.5 559.9	585.7	98.2	N N	0 1
Least Difference[3]						49.9	10.9		

[1] Histological examination shows rickets.
[2] Histological examination shows normal bone calcification.
[3] The least difference between any two means required to give odds of 19:1. In this and subsequent tables, the average of each lot of birds is considered as a unit for statistical treatment, thus the value of each unit for the purpose of analysis is much greater than would at first appear from the use of two or three units on each ration. The method of analysis corresponds to Example 3 and the interpretation is based on Example 4 of Snedecor, G. W., Calculation and Interpretation of Analysis of Variance and Covariance. Collegiate Press, Inc., Ames, Iowa, 1934, p. 21–34.

the moisture and fat-free right tibia bone of each chick was determined.

RESULTS

The summary of the 1935 experiment at the eight-week period is reported in Table 3. The average feed consumed per chick was considerably higher when adequate quantities of vitamin D were included in the ration. The grams of feed required per gram of gain were greater in the lots affected with rickets than in the lots that had sufficient vitamin D to prevent rickets. There was no significant difference in growth between the positive control lots and the lots fed the two high levels of salmon fish meal.

The histological examination was made of the left tibia of each chick to determine the degree of calcification, as described by Carver, Brunstad, St. John, Frasier, and Athow (1933). All chicks in the negative control lots, and the lots fed the 0.7 percent and the 1.4 percent salmon fish meal were affected with rickets. There were no chicks in the positive control lots or the lots fed 2.9 percent and 5.7 percent levels of salmon fish meal affected with rickets. Only three chicks died in the entire experiment, two of rickets in the negative control lots, and one of accident in the high level salmon fish meal lot.

The relative growth of chicks as a measure of the vitamin D value of the salmon fish meal is illustrated in Figure 1. The average body weight at eight weeks of the positive control, lots 2 and 8, was assumed to have a value of 100 percent. The negative control duplicate lots had an average weight of the positive control of 43.9 percent; the 0.7 percent salmon fish meal, 54.9 percent; and the 1.4 percent salmon fish meal, 70.3 percent. The duplicate lots fed 2.9 percent salmon fish meal showed a significant difference over the lots fed 1.4 percent salmon fish meal, with an average weight of the positive control of 93.8 percent. The duplicate lots fed 5.7 percent salmon fish meal showed no significant difference in the average weight of the positive control over the lots fed 2.9 percent salmon fish meal.

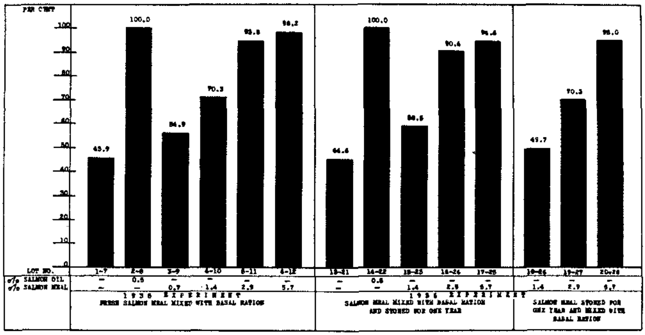

FIG. 1. The relative growth of chicks as a measure of the vitamin D value of stored salmon fish meal.

TABLE 4.—*Summary of 1936 results at eight weeks*

Lot No.	Date of mix	Vitamin D carrier fed	Source of vitamin D	Av. feed consumed per chick	Grams of feed per gram of *gain*	Average chick weight	Av. wt. of chicks in duplicate *lots*	Av. wt. of positive control	Degree of calcifica-*cation*	Bone ash analysis	Average bone ash *analysis*	Mortality
		percent		grams		grams	grams	percent		percent	percent	
13 21	Jan. 1935	0.0	None	902.7 964.1	4.5 4.5	239.2 253.0	246.1	44.6	R R	40.9 41.7	41.3	1 5
14 22	Jan. 1936	0.5	Salmon fish oil	1566.0 1589.1	3.1 3.1	550.0 553.1	551.5	100.0	N N	58.6 49.5	49.1	0 0
15 23	Jan. 1935	1.4	Salmon fish meal	1152.8 1168.7	4.0 4.1	325.0 320.8	322.9	58.5	R R	43.8 43.4	43.6	0 0
16 24	Jan. 1935	2.9	Salmon fish meal	1453.2 1534.6	3.2 3.3	495.4 501.7	498.5	90.4	N N	45.8 47.9	47.0	0 0
17 25	Jan. 1935	5.7	Salmon fish meal	1472.0 1539.6	3.0 3.2	525.8 517.3	521.5	94.6	N N	49.9 48.6	49.2	0 0
18 26	Jan. 1936	1.4	Salmon fish meal	978.9 1058.4	4.2 4.4	270.8 277.3	274.0	49.7	R R	43.5 42.9	43.2	1 0
19 27	Jan. 1936	2.9	Salmon fish meal	1295.0 1295.4	3.5 4.0	410.4 365.4	387.9	70.3	R R	45.4 44.2	44.8	0 0
20 28	Jan. 1936	5.7	Salmon fish meal	1517.4 1559.9	3.1 3.2	527.5 520.5	524.0	95.0	N N	48.2 47.4	47.9	1 0
Least Difference							30.4	7.3				

Table 4 summarizes the results obtained in the second year's experiment after a year's storage of the feed. The lots receiving 0.7 percent salmon fish meal were omitted in the second year's experiment since this level was shown in the first experiment to be below the requirements for protection against rickets. As in the previous year's experiment, the average feed consumed per chick was considerably higher when adequate quantities of vitamin D were included in the ration. The grams of feed required per gram of gain also were greater in the lots affected with rickets than in the lots that had sufficient vitamin D to prevent rickets.

There was no significant difference in growth between the positive control lots and the lots fed the 5.7 percent salmon fish meal. All other lots showed a significant difference in growth when compared with the positive control lots.

The histological examination of the left tibia of each chick showed that all chicks in the negative control lots, the lots fed the 1.4 percent salmon fish meal mixed in 1935 and 1936, and the 2.9 percent salmon fish meal lots mixed in 1936 were affected with rickets. There were no chicks in the positive control lots or the lots fed 5.7 percent salmon fish meal mixed in 1935 and 1936 or the lots fed 2.9 percent salmon fish meal mixed in 1935 affected with rickets. Only eight chicks died in the entire experiment, six of which died of rickets in the negative control lots.

The results in bone ash analysis obtained in the second year's experiment showed that the tibiae of lots 14 and 22, 16 and 24, 17 and 25, and 20 and 28 were well calcified. The tibiae were poorly calcified and showed definite evidence of rickets in lots 13 and 21, 15 and 23, 18 and 26, and 19 and 27. The relation of the percent bone ash in the tibiae to the vitamin D in the ration agrees with the results reported by St. John, Kempf, and Bond (1933) and Bethke and Record (1934). Lots 19 and 27 were significantly below lots 16 and 24.

The relative growth of chicks as a measure of the vitamin D value of stored salmon fish meal is shown in Figure 1. The average body weight at eight weeks of the positive control lots 14 and 22 was assumed to have a value of 100 percent. The negative control lots had an average weight of the positive control of 44.6 percent. Where the salmon fish meal was stored for one year mixed in the ration, the lots fed the 2.9 percent and the 5.7 percent salmon fish meal were 90.4 percent and 94.6 percent of the average weight of the positive control. When the salmon fish meal was stored separately for one year and then mixed in the ration, the lots fed the 5.7 percent salmon fish meal were 95.0 percent of the average weight of the positive control. The lots fed 2.9 percent salmon fish meal were only 70.3 percent of the positive control in weight as compared to 90.4 percent when the salmon fish meal was stored for one year mixed in the ration. The lots fed the 5.7 percent salmon fish meal stored separately and mixed in the ration in 1936 showed a significant difference in the average weight of the positive control over the 2.9 percent level of salmon fish meal. There was no significant difference between the average weight of the positive control of the 5.7 percent salmon fish meal lots stored for one year mixed in the ration and the lots fed the same level of salmon fish meal stored separately for one year and mixed in the ration.

SUMMARY

The 2.9 and the 5.7 percent levels of fresh Columbia River salmon fish meal supplementing the all-mash chick ration supplied complete protection from rickets.

The 2.9 and the 5.7 percent levels of Columbia River salmon fish meal when stored for one year mixed in the all-mash

chick ration supplied complete protection from rickets.

After the Columbia River salmon fish meal had been stored separately for one year and then mixed with the all-mash chick ration, it was found that only the 5.7 percent level supplied the chicks complete protection from rickets.

It was found that when salmon meal was mixed with the basal ration and stored for one year that the vitamin D value was greater than from salmon meal stored under the same conditions separately. From these results it is not improbable that the basal ration could have acted as an antioxidant in preventing losses of vitamin D during the storage of the salmon fish meal.

As a result of these experiments, it would appear that high fat salmon fish meals can be manufactured and stored for at least a year and be satisfactorily used if fed at sufficiently high levels to supply the vitamin D in the poultry ration. It is apparent that further work is needed on the methods of storage of fish meals.

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The Effect of Feeding Grit on Digestibility in the Domestic Fowl

James C. Fritz

Bureau of Animal Industry, United States Department of Agriculture, Washington, D.C.

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THE American literature on the value of poultry grit has been well reviewed by Waite (1935), and his conclusions can be summarized in a few statements. Most of the work on the subject has consisted of comparing the growth, egg production, and feed consumption of one group of birds receiving grit with those of another group which did not receive grit. It has been definitely proved that birds can be successfully raised and maintained without access to any grit. However, several workers have observed that birds without grit consumed more feed per unit gain in weight.

Danilova, Solun, Chlebnikov, and Poliakov (1933) reported that the addition of grit did not improve the digestibilty of crude fiber, but that it did increase the digestibility coefficients for protein, fat, and carbohydrates.

The question of the effect of grit on the utilization of different feeds has received little attention. It is sometimes assumed that the bird needs grit when whole grain or other coarse, hard feed is given, and that the bird does not need grit when an all-mash diet is fed. Kath (1925) removed the grit from the gizzards of a number of birds by a surgical operation and then compared the quantity of feed required for maintenance with that required by a similar, normal control group. He found that the birds could maintain their weight on either hard or soft feeds when they had no grit in their gizzards; but that the birds without grit consumed more of either hard or soft feed than did the control birds with grit.

The object of the present study was to determine the effect of grit on the bird's ability to digest its feed. The influence of grit, if any, must be one of aiding digestibility. In order to compare all-mash feeds and coarse granular feeds, digestibility trials were made with a modification of the normal all-mash laying diet used at this station, and with southern field peas. One reason for using the peas was to obtain additional information on the feeding value of this legume. Southern field peas are used to a considerable extent in pigeon feeding and at the usual market price are an inexpensive source of protein. The peas used in this experiment were of the Whippoorwill variety.

MATERIALS

The all-mash diet had the following composition:

	Percent
Ground yellow corn	35.30
Wheat bran	16.75
Rolled oats	10.25
50% meat and bone scrap	7.20
N.A. white fish meal	6.30
Dried skimmilk	4.50
Dried whey	4.50
Alfalfa leaf meal	3.75
Limestone flour	3.70
Yeast	2.50
Steamed bone meal	2.35
Cod liver oil	2.00
Anhydrous sodium sulfate	0.45
Sodium chloride	0.45

The chemical composition of the feeds is shown in Table 1.

The grit used in these tests was a granite grit from Georgia. An insoluble grit was chosen to avoid any chance of rapid solution of the grit by acid secretions in the digestive tract. Ferber and Brüggemann

(1933) have reported much better results with a granite grit than either without grit or with a limestone grit. However, Waite (1935) found that granite grits in general, and this Georgia product in particular, shattered very readily. Waite considered this type of grit inferior to native pebbles. Two sizes of grit were obtained: a "baby chick" size which included particles up to about 2 mm. diameter, and averaged about 1 mm. diameter; and a "turkey" size with particles varying from 6 mm. to about 14 mm. diameter.

Two adult male birds were used in these tests. One was a Rhode Island Red and the other was a crossbred bird. These birds were removed from the incubator and immediately placed on a wire mesh floor. They were never allowed access to any grit. All mineral supplements in their diet were very finely ground. Other birds from the same source were killed and examined. Their gizzards did not contain any hard particles. At eight months of age these birds were altered by surgical means so that the urine and the feces were voided separately. This was done by bringing the colon to the surface about two inches below the cloaca. This operation has been described in detail by Paraschtschuk (1902), Katayama (1924), Maas (1933), and others. Katayama (1924) has shown that this operation does not change the bird's ability to digest its feed. Two months elapsed between the time of the operations and the beginning of the digestion trials.

TABLE 1.—*Chemical composition of the feeds*

Diet	Moisture	Crude protein (total N $\times$6.25)	True protein (albuminoid N$\times$ 6.25)	Non-protein N compounds	Ash	Ether extract	Crude fiber	N-free extract
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
All-mash laying diet	8.82	17.54	16.40	0.86	8.27	9.66	2.41	53.58
So. field peas (Whippoorwill)	13.65	22.27	20.09	1.64	2.90	1.52	4.58	55.62

PROCEDURE

Each bird was placed in an individual wire cage 26 inches high, 21 inches long, and 17 inches wide. The feeds were alternated in successive trials, i.e., the field peas were used in the first trial, then the all-mash diet was fed during the second trial, field peas during the third trial, and so forth. After four digestion trials without grit, two with the peas and two with the all-mash diet, baby-chick-size granite grit was fed and the same number of digestion trials with the peas and the all-mash diet were repeated. Finally, turkey size granite grit, from the same source, was fed and the digestion trials were again repeated with the peas and with the all-mash diet. During the trials with grit, a daily consumption of from three to six grams of grit was maintained by limiting the amount of grit before the birds to six grams each, and by force-feeding the grit if too little was eaten voluntarily.

The birds ate both diets readily. The daily intake of the peas was limited to 50 grams per bird per day after it was observed that the intake of larger quantities sometimes caused feed to remain in the crop over night.

The peas were set before the birds in open dishes and the birds allowed to eat under close observation to guard against any waste. Any of the 50 grams of peas not eaten voluntarily in the morning was again placed before the bird in the afternoon. Any peas still refused by the bird were force-fed. The birds ate the peas without any need for force-feeding in nearly every case. The all-mash diet was placed before the birds in containers with a hood over the top and the sides and with a lip inside the cup. This made it almost impossible for the birds to waste any feed. In addition, the birds had the feed before them only as long as they ate steadily, after which the containers were removed and the residue of the feed weighed. Consumption of the all-mash diet was voluntary and the daily consumption averaged about 70 grams per bird per day.

Enemas were given twice daily, at 9:00 a.m. and at 3:30 p.m. At all other times a glass plug was kept in the artificial anus. The samples collected by means of the enemas at 3:30 p.m. one day and at 9:00 a.m. on the following day were combined. This combined sample was mixed with 10 c.c. of 5 percent hydrochloric acid (by volume) and then dried on a boiling water bath. Records of the daily weight of the feces were kept. After the daily excretion became constant in weight, usually after three or four days on the experimental diet, the feces samples for eight days were collected and combined.

Samples of the feeds and the feces were analyzed by official methods (Association of Official Agricultural Chemists [1930]), for moisture, crude protein, true protein, ash, ether extract, and crude fiber. Organic matter, non-protein nitrogen compounds, and nitrogen-gree extract were calculated from the values for the other constituents. From the weight and composition of the feed and feces, the digestibility coefficients were calculated for each of these constituents.

EXPERIMENTAL DATA

Average variations in the live weights of the birds during the course of the digestibility trials are shown in Table 2. The average coefficients of apparent digestibility are shown in Table 3. No attempt was made to calculate the digestibility of mineral matter because of the obvious variations due to changes in feeding.

TABLE 2.—*Variations in live weight during the digestion trials*

Feed	Average gain or loss in weight during trial		
	Without grit	With fine grit	With coarse grit
	grams	grams	grams
So. field peas	−79	−165	−55
All-mash diet	−31	+ 56	+25

DISCUSSION

The field peas did not constitute a complete diet suitable for birds kept in strict confinement. It is not surprising that the birds lost considerable weight during all trials when the peas were fed. It is interesting to note that the birds lost weight on the all-mash diet when they did not receive grit and that they gained weight when they received the all-mash diet supplemented with either fine or coarse grit.

The coefficients of apparent digestibility for most constituents were slightly higher when grit was fed. The values obtained for the digestibility of non-protein nitrogen compounds and for crude fiber were rather variable and of minor importance. However, it was apparent that the birds did utilize more of the non-protein nitrogen than popular opinion would indicate.

The most significant difference in digestibility was noted in the case of the ether extract. Fritz (1935) observed that grinding

flint corn before feeding resulted in a significantly higher digestibility of fat. Apparently, grinding of the feed is essential if a high utilization of fat is desired, and the bird can do a more satisfactory job of grinding the feed if there is grit in the gizzard. These results are in agreement with those reported by Danilova, Solun, Chlebnikov, and Poliakov (1933).

The value of grit was more apparent when the field peas were fed. Evidently there is more need for grit when coarse, granular feeds are used. Some crude fiber was utilized when grit was given but the crude fiber of the peas was not digested without grit.

There was no consistent difference in digestibility coefficients obtained with the feeding of the two sizes of grit. In most cases there was slightly better utilization of

TABLE 3.—*Coefficients of apparent digestibility.*

Feed	Grit	Coefficients of apparent digestibility							
		Dry matter	Organic matter	Crude protein (total N ×6.25)	True protein (albuminoi N×6.25)	Non-protein N compounds	Ether extract	Crude fiber	N-free extract
All-mash diet	None	77.78	82.33	81.57	83.75	67.27	87.12	33.71	84.12
		81.25	84.96	86.18	88.18	73.18	86.84	38.96	86.33
		76.61	82.33	78.75	82.21	55.99	85.92	32.48	85.56
		84.63	86.74	88.07	89.62	77.89	90.80	34.84	88.00
	Av.	80.04	84.09	83.64	85.94	68.58	87.67	35.00	86.00
	3–6 gms. per day fine granite grit	81.05	86.53	84.50	86.88	68.86	93.15	38.93	88.45
		80.85	85.49	86.58	88.26	75.52	92.57	44.20	86.08
		82.49	86.22	85.30	86.87	74.92	91.65	47.77	84.92
		80.60	86.04	87.15	88.91	75.64	88.97	47.67	86.87
	Av.	81.25	86.07	85.88	87.73	73.74	91.59	44.64	86.58
	3–6 gms. per day coarse granite grit	82.65	86.81	86.07	87.73	69.47	90.82	49.30	88.20
		80.72	85.96	87.12	88.68	76.49	91.42	41.64	86.63
		86.46	87.68	85.73	88.08	52.02	92.18	35.46	89.67
		84.04	86.89	86.78	88.99	55.13	92.58	23.87	88.56
	Av.	83.47	86.84	86.42	88.37	63.28	91.75	37.57	88.27
Southern field peas (Whip-poorwill variety)	None	64.82	65.69	70.11	80.58	9.21	66.80	Negative	75.23
		68.78	71.81	76.67	82.10	26.62	70.31	Negative	78.06
		62.52	63.33	66.40	74.43	0.00	71.67	Negative	70.96
		69.59	70.83	70.09	76.16	14.38	52.69	Negative	79.52
	Av.	66.43	67.92	70.82	78.32	12.55	65.37	Negative	75.94
	3–6 gms. per day fine granite grit	63.61	75.12	77.77	79.33	63.37	83.65	24.09	78.26
		70.99	75.53	74.32	79.97	22.12	83.19	14.34	80.72
		68.10	74.73	73.33	76.31	45.96	73.36	20.30	79.89
		73.46	73.32	75.32	80.22	32.87	77.11	9.12	78.82
	Av.	69.04	74.68	75.19	78.96	41.08	79.33	16.96	79.42
	3–6 gms. per day coarse granite grit	75.90	74.94	74.15	78.74	29.01	76.52	14.74	80.09
		73.45	75.68	77.76	82.06	38.16	83.88	13.12	79.80
		74.36	74.75	74.24	78.75	32.60	76.61	9.49	80.29
		75.24	76.27	76.87	81.11	37.81	81.92	10.85	81.29
	Av.	74.74	75.41	75.76	80.17	34.40	79.73	12.05	80.37

the nutrient materials when the coarse grit was fed.

The digestibility of the southern field peas was sufficiently good to justify the use of these legumes in poultry feeds. Although the coefficients of apparent digestibility for the peas were much lower than the usual coefficients for corn, the peas contained approximately 60 percent more digestible protein per unit of weight.

SUMMARY AND CONCLUSIONS

Artificial ani were prepared in two male birds which had been raised to maturity on screen floors and without access to any grit. Digestibility trials were conducted on southern field peas (Whippoorwill variety) and on an all-mash laying diet. The experiments were repeated on the same feeding stuffs with the birds fed fine granite grit. Finally, the digestibility trials were repeated with the birds fed a large size granite grit. In general, the effect of feeding grit was slight. The digestibility of all constituents was slightly improved. The improvement was more noticeable in the digestibility of fat. The influence of grit on digestibility was greater when coarse feed was used. The digestibility of the southern field peas was considered sufficiently good to justify their use in poultry feeds.

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